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PHYSIOLOGICAL CHANGES IN THE RIND OF CALIFORNIA ORANGES DURING GROWTH AND STORAGE[1]

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INTRODUCTION

This paper deals with the changes that normally occur in the rind of Valencia and Washington Navel oranges (*Citrus sinensis* (L.) Osbeck) both while the fruit is on the tree and after it is picked and held in storage at various temperatures. The investigation formed part of a general physiological study of the rind of citrus fruits with particular reference to the cause or causes of certain rind pitting and spotting diseases that occur in transportation and storage.

When the work was begun it was deemed desirable to place the greater emphasis upon the determination of the normal changes in the rind that occur while the fruit is on the tree. However, even at that time, a few collections were taken for storage study. Emphasis was not shifted to the determination of changes in the rind under various storage conditions until considerably later. Therefore, this paper is concerned almost entirely with the rind of Valencia and Washington Navel oranges in the field, and the observations on the effects of cold storage are to be considered preliminary.

Although the results of this investigation allow the comparison of the Washington Navel and Valencia orange rinds in certain aspects, it should be kept in mind that under the circumstances much more than varietal characteristics must be considered. One of the most important factors involved is the difference in the season of the year in which the two varieties are harvested. This phase of the investigation is considered in the section of this paper dealing with a comparison of the Washington Navel and the Valencia orange rinds.

REVIEW OF LITERATURE

In previous papers on oranges a preponderant amount of attention has been given to the pulp and juice. The reason for this is readily understood, but when one comes to deal with an important disease that has its seat entirely within the rind, the physiology of this tissue assumes a new interest. It is true that much is known regarding the essential oils of the rind, but relatively little is known of its other constituents, particularly in relation to their seasonal variations in the field or in storage.

Bigelow and Gore (*1*)[2] observed changes in the proportion of rind to pulp of oranges during ripening, and later Copeman (*3*) made

[1] Received for publication Aug. 30, 1935; issued June 1936.
[2] Reference is made by number (italic) to Literature Cited, p. 746.

similar observations through a period of 104 days. Both of these papers record a small but constant decrease in the proportion of rind to pulp as the season advances. Willimott and Wokes (*8, 9*) reported an extensive study of seasonal distribution of vitamins, oxidases, sugars, and acid in the pulp and rind of citrus fruit. Recently Gaddum (*4*) has followed the seasonal variation of the pectins in Valencia oranges. He found that the pectic compounds remained practically constant in the albedo and pulp until after the full-ripe stage, when they began to decline; and that the water-soluble pectins increased to a maximum just previous to the beginning of the decline of the pectic compounds, after which they also began to decline—a situation which is of interest in connection with the late-season increase of susceptibility to storage spot often observed. Many other papers are concerned either wholly or in part with the orange rind, but most of the experiments reported were not designed to show seasonal behavior. As a guide to these works, the extensive bibliography of the genus *Citrus* compiled by Matlack [3] is recommended.

MATERIALS AND METHODS

The study reported herein was carried on through the orange seasons of 1932 and 1933 in two localities, Pomona and Corona, Calif.[4]

Collections could not be made from the two localities on the same day because of the time required in the laboratory to give each the necessary immediate care. Usually the Corona collection was made 1 day after the Pomona collection, but in December and January there was a difference of 4 days; and in both instances a rise of temperature occurred immediately following the collection at Pomona. As a result the means for the 5-day periods preceding the corresponding collections at Corona were several degrees higher than they would have been had the pickings been made the next day after the ones at Pomona.

Collections were always made in the early morning. Each field collection consisted of 50 fruits, or 10 from each of 5 selected trees, taken with as uniform spacing as possible. When the fruit arrived in the laboratory the rinds were removed at once and each rind was divided into six portions as follows: (1) Stem-end flavedo;[5] (2) stem-end albedo, (3) equatorial flavedo, (4) equatorial albedo, (5) blossom-end flavedo, and (6) blossom-end albedo. A separation of this sort was considered desirable on account of the frequent localization, especially in the Valencia orange, of pitting and spotting in more or less specific portions of the rind. This arrangement allowed the making of four major comparisons, namely: (1) Seasonal, as regards the rind as a whole, and both general and seasonal of (2) stem and blossom ends, (3) flavedo and albedo, and (4) fruit from the two localities. The two equatorial portions were analyzed exactly as were the stem-end and blossom-end portions, but the data are not considered necessary for this report and are not presented, unless specifically mentioned. The quantities recorded for the equatorial portions are almost always intermediate in value between the corresponding ones in the stem and blossom ends.

[3] MATLACK, M. B. BIBLIOGRAPHY ON THE CHEMISTRY OF THE GENUS CITRUS. U. S. Dept. Agr., Bur. Chem. and Soils. 159 pp. 1932. [Mimeographed.]

[4] These two localities are about 30 miles apart and in the same general district. The grove used in Corona was at the southeastern side, and the one in Pomona was near the northern side of the district. The climate was approximately the same for each grove, although at Corona the temperatures were somewhat higher and there was more sunshine and less humidity.

[5] The terms "flavedo" and "albedo" designate outer rind and inner rind, respectively.

Water was determined in fresh lots which were separated from the general macrochemical sample just previous to the preservation of the latter in alcohol. Two methods were employed: (1) The usual drying of the tissue to constant weight and (2) the toluene method. The latter method, in spite of its known faults, was found to be the more satisfactory for the flavedo because of the error introduced by oven drying, due to the high volatile-oil content of this outer rind tissue.

The general macrochemical samples consisted of 50 g fresh weight, and these, as just indicated, were preserved by covering them with 95-percent alcohol and heating to 70° C. for 1 hour.

Nitrogen and carbohydrates were determined by the methods described by Harvey (*6, pp. 8, 9*).

The sugars were calculated as follows: (1) "Reducing sugar", as glucose, from the copper equivalent to the permanganate titration before inversion; (2) "invert sugar", as invert sugar, from the copper equivalent to the permanganate titration after inversion minus the permanganate titration before inversion; and (3) "total sugar", as the sum of "reducing sugar" plus "invert sugar."

Hesperidin was determined in the rind of the Washington Navel variety only. The method of analysis was as follows: Into a 300-ml Florence flask were transferred 25 ml of the extract, or soluble fraction, 51 ml of water, and 4 ml of concentrated hydrochloric acid. This mixture was allowed to simmer for 40 minutes under a reflux condenser. The contents of the flask were then cooled, neutralized, and filtered.

In the early analyses hesperidin was determined by two methods: (1) By collecting the insoluble derivative, hesperitin, on a Gooch filter, then drying and weighing; and (2) by determining the increased reducing power after hydrolysis. Both methods were tested with pure hesperidin solutions as well as with tissue extracts. The gravimetric method, although satisfactory otherwise, required such large samples (200 ml of extract) to obtain sufficient accuracy that it was finally abandoned in favor of the other method, that is, the determination of the sugars derived from hesperidin. The weight of the sugars found was multiplied by the factor 2.17. This is larger than the theoretical factor, but it was found that the destruction of sugar during hydrolysis made this modification necessary.

Hydrogen-ion determinations were made on the rind of Washington Navel oranges by means of a Leeds and Northrup "student" potentiometer with a quinhydrone electrode. Juice from the different rind portions was obtained by means of a specially constructed press after the tissue had been frozen for 24 hours at temperatures of −15° to −20° F. Flavedo tissue readily yielded abundant juice, but it was often difficult to express sufficient quantities of juice from the amount of albedo tissue available.

In making the hydrogen-ion determination it was found that the potential of the rind juice showed a strong tendency to drift during readings. This difficulty was largely overcome by using a large excess of quinhydrone and by diluting the juice to five times its original volume. At this dilution the pH was approximately the same as that of the undiluted juice, whereas a lesser dilution had a tendency to give a smaller pH and a greater dilution a larger pH than the original.

In each locality five adjacent trees of Valencia oranges were selected to furnish the experimental material. The trees at Pomona were 28 years old and were growing in Hanford gravelly sandy loam. One-

half of each tree was irrigated every 2 weeks. At Corona the trees were within a year of the same age and were growing in Yolo gravelly loam. The whole of each tree was irrigated about every 30 days.

From May 18 to November 14, 10 collections of fruit were made from each locality. The results indicated later that the first collection probably should have been made about May 1 or April 15 instead of May 18 when the fruit was already well colored. The interval between collections was 2 weeks except the last three intervals, which were approximately 30 days. In November additional lots were collected from each locality and stored, some at 33° and some at 53° F. These were held for 7 weeks for observation and analysis.

The Washington Navel oranges were from similarly selected trees in the same localities. The trees in the Pomona grove were 43 years old and those in the Corona grove were 30 years old.

The intervals between collections varied with the season as follows: Those between collections nos. 1 and 2, and 2 and 3 were 3 weeks; those between nos. 3 and 4, and 4 and 5 were 4 weeks; and the interval between nos. 5 and 6 was 6 weeks.

Additional lots of fruit were collected from each locality for storage at 52°, 42°, and 32° F. These collections corresponded with the field collections of January (no. 3), March (no. 5), April (no. 6), and the extra Pomona field collection.

Six regular field collections were made during a period of 140 days beginning November 25, 1932, and ending April 13, 1933. The first collection was made before the usual commercial picking time, when the fruit was still dark green, and the last was made after the regular picking season. From the first the Pomona fruit was somewhat deeper in color than the Corona fruit, and as the season advanced it became evident that the latter was maturing more rapidly than the former. By the first week in January the fruit at both locations was well "colored." One extra field collection was made at Pomona on May 25 to serve as a control for a storage experiment that was started on that day. Since the last collection date at Corona coincided with the time of full bloom, the fruit was falling readily and showed some lack of firmness. On the corresponding collection date at Pomona the fruit was still firm, and the trees were not in full bloom until May 25. At the last, or extra, collection at Pomona the trees were blossoming and the fruit was much like that at Corona on the previous collection date.

EXPERIMENTAL RESULTS

Most of the experimental data reported herein were obtained under field conditions, but some were obtained under conditions of storage.

CHANGES UNDER FIELD CONDITIONS

Seasonal Changes

The chemical data are summarized in table 1, which gives the average values of the different substances under observation. These averages were calculated from the combined results of all portions of the rind and from both localities for each collection date; for Valencias each value is the average of 24 analyses and for Washington Navels it is the average of 16 similar analyses.[6]

[6] Duplicate samples were analyzed from each of the two localities; with the Valencia rinds there were six portions, but with the Washington Navels only four portions since the equatorial portions were not analyzed.

TABLE 1.—*Seasonal changes in the chemical composition of orange rinds*[1]

TABLE 1.—*Seasonal changes in the chemical composition of orange rinds* [1]

Variety	Date of collection [2]	Reducing sugar	Invert sugar	Total sugar	Soluble solids	Insoluble solids	Hydrolyzable polysaccharides	Soluble nitrogen	Insoluble nitrogen	Total nitrogen	Water	Flavedo-albedo ratio	Hesperidin	Hydrogen-ion concentration
	1932	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*		*Percent*	*pH* [3]
Valencia	May 21	20.11	9.18	29.29	48.57	41.36	13.93	0.384	0.466	0.850	72.0			
	June 4	21.66	9.78	31.44	54.35	36.09	11.92	.392	.444	.836	72.4	1.57		
	June 18	21.36	10.07	31.43	53.57	37.89	12.96	.379	.461	.840	72.0	1.57		
	July 2	20.37	10.66	31.03	52.75	37.73	12.71	.405	.477	.882	72.7	1.59		
	July 16	19.06	10.18	29.24	51.62	36.82	12.20	.464	.468	.932	72.3	1.63		
	July 30	19.45	11.69	31.14	51.84	39.74	13.66	.435	.484	.919	72.8	1.60		
	Aug. 13	18.66	11.34	30.00	51.12	37.61	12.43	.426	.468	.894	73.2	1.66		
	Sept. 10	15.35	13.35	28.70	50.77	39.42	13.23	.456	.495	.951	74.0	1.77		
	Oct. 8	17.78	10.56	28.34	51.98	36.55	11.79	.463	.465	.928	74.8	1.98		
	Nov. 12	20.74	7.71	28.45	54.61	36.27	11.48	.426	.430	.856	72.9	1.73		
	Nov. 25	24.02	5.71	29.73	52.99	47.01	15.35				75.9	1.60	3.55	5.38
	Dec. 16	27.93	6.09	34.02	57.47	42.53	13.51				74.5	1.04	3.70	5.62
Washington Navel	*1933*													
	Jan. 5	30.37	4.76	35.13	56.49	43.51	14.22				72.2	1.12	3.07	5.61
	Feb. 1	31.56	6.63	38.19	60.17	39.83	13.28				73.1	1.02	2.91	5.53
	Mar. 1	34.20	6.35	40.55	61.99	38.01	12.55				72.4	1.02	2.20	5.89
	Apr. 12	34.85	6.27	41.12	62.95	37.05	11.93				74.6	1.02	2.03	5.73

[1] All values except water and hydrogen-ion concentration are calculated on the basis of dry weight.

[2] Collections in the 2 localities were 3 days apart, the Pomona collection always preceding the Corona. An intermediate day is used in this table for convenience.

[3] The writers are fully aware that the averaging of pH values, here and elsewhere in this paper, is mathematically unsound, but they believe that through the ranges shown the averages of pH values as numerical entities are probably as sound biologically as the averages of the actual hydrogen-ion concentrations or the equivalent pH values, and are considerably more convenient.

Water.—In such a summary of data as that given in table 1 it does not appear at a glance that there was really a seasonal trend of water content; but on closer examination and in consideration of the number of analyses represented, it becomes clear that in Valencia oranges there was a gradual rise of water nearly to the end of the season (fig. 1). After that time there was a slight decrease of water, as recorded in the last collection. In the data from individual portions of the rinds (data which cannot be presented here on account of limited space) some considerable fluctuations of water were noted. However, all these larger variations seemed to be correlated chiefly with the availability of soil moisture, although there was some correlation with temperature and other environmental factors, but not with the

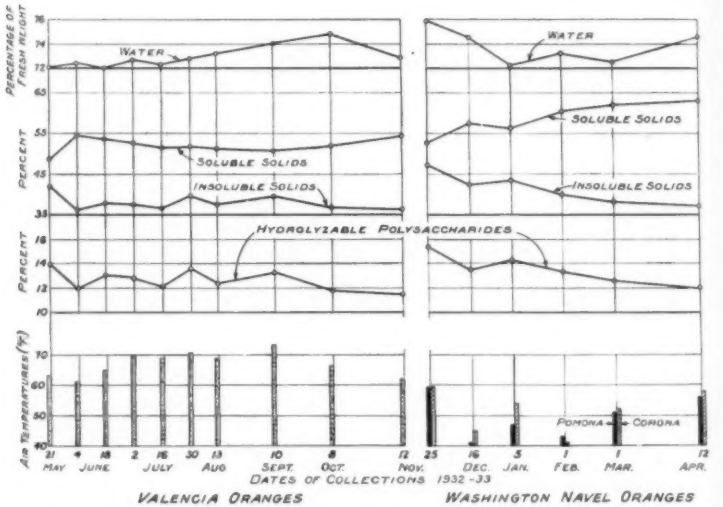
PERCENTAGE OF FRESH WEIGHT
WATER
SOLUBLE SOLIDS
INSOLUBLE SOLIDS
HYDROLYZABLE POLYSACCHARIDES
AIR TEMPERATURES (°F)
POMONA
CORONA
21 4 18 2 16 30 13 10 8 12 25 16 5 1 1 12
MAY JUNE JULY AUG SEPT. OCT. NOV. DEC. JAN. FEB. MAR. APR.
DATES OF COLLECTIONS 1932-33
VALENCIA ORANGES
WASHINGTON NAVEL ORANGES

FIGURE 1.—Seasonal changes in mean air temperature (means for 5-day periods preceding collections), water, soluble and insoluble solids, and hydrolyzable polysaccharides content of the rind of Valencia and Washington Navel oranges.

maturity of the fruit. That is to say, the water-holding power of orange rinds changed only slightly with the season. The water content was not correlated closely with mean temperature, possibly because all collections were made early in the morning, when the saturation deficit set up during the previous afternoon may have been mostly satisfied.

The magnitude of this possible diurnal variation of water content in Valencia rinds was investigated through a period of 24 hours. Six collections were made during a night and day following a hot day. The largest diurnal difference between midafternoon and early morning was about 2 percent, but this value is probably not very close to the maximum variation produced by the occasional severe conditions encountered in orange groves during the summer.

In Washington Navel oranges the water content showed a downward trend during the early part of the season, followed by fluctuations due to immediate environmental factors (fig. 1).

Soluble solids.—In Valencia oranges, as shown in figure 1, the soluble solids in the rinds as a whole vary almost inversely with the mean temperature.[7] There is a broad minimum during August-September, corresponding to the hottest weather of the season, and two maxima, one in June and the other in October-November, the coolest periods. The separate portions of the rinds often showed greater fluctuation but in the same direction. The higher temperatures and the lower water content evidently favored the condensation of sugars in the rinds to insoluble polysaccharides, hence the lower soluble solids. In Washington Navel oranges, on the other hand,

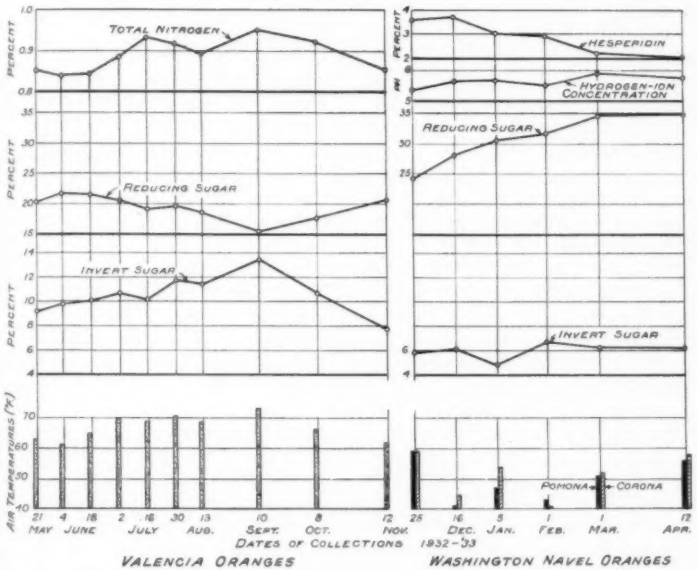

FIGURE 2.—Seasonal changes in mean air temperature (means for 5 day periods preceding collections), reducing and invert sugar, total nitrogen, hydrogen-ion concentration, and hesperidin content of the rind of Valencia and Washington Navel oranges.

there was an increase in soluble solids and a decrease in insoluble solids as the season advanced.

Hydrolyzable polysaccharides.—In Valencias the polysaccharides graph (fig. 1) shows a strong tendency to parallel the mean-temperature graph. When temperatures were high the polysaccharides were high, and vice versa. Individual portions of the rind showed this tendency more strikingly than did the rind as a whole. In Washington navels the graph for polysaccharides practically parallels that for insoluble solids.

Total sugar.—In Valencia oranges total sugar showed little change through the season, owing to the fact that the behavior of the reducing sugar was almost the reverse of that of invert sugar on all

[7] The mean-temperature graph was constructed by averaging the mean temperature of the 5 days immediately preceding each collection date at each locality. The two graphs resulting were essentially alike throughout, that of Corona being somewhat the higher.

occasions: if reducing sugar was high, invert sugar was low, and vice versa. The graph in figure 2 representing reducing sugar is somewhat similar to that of soluble solids (fig. 1). The invert-sugar graph (fig. 2), on the other hand, nearly parallels the mean-temperature graph.

The seasonal trend of reducing sugar is of considerable interest. There are two maxima and one minimum. The first maximum occurred at the second collection; it may have marked the end of the relatively rapid development of the orange toward maturity. As the season advanced the general level at which reducing sugar could be maintained (without being condensed to sucrose or other higher carbohydrates) became lower and lower, reaching a minimum during the hottest weather. This minimum perhaps marked a stage just previous to rapid deterioration. It was the time when the rind was freest from green color, and it coincided with the invert-sugar maximum. After this period there was an apparent reversal of the processes and the rinds began to "go back" in color, or become greener, particularly at the stem end. The reducing sugar increased and the invert sugar decreased, so that, as the temperatures dropped in autumn nearly to those of April or early May, the chemical picture within the Valencia rinds became similar in this respect to the one early in the season.

In the Washington Navel variety total sugar increased continually through the season, largely because of the steady increase in reducing sugar (fig. 2). Invert sugar fluctuated apparently more under the influence of immediate environmental factors. The graph of invert sugar is somewhat the inverse of the mean-temperature graphs, although there was apparently a very small increase as the season progressed.

Nitrogen.—There were no large seasonal changes in the percentage of total nitrogen, which was determined only in the Valencia variety. The individual portions of the rind showed numerous apparent fluctuations not only in the percentage of but also in the relations between soluble and insoluble nitrogen. Whether the total quantity of nitrogen actually changed is difficult to determine on account of the much greater variations of other substances. There must have been changes in the form, because, for example, the nitrogen released upon the decomposition of chlorophyll seemed to have remained in the rind; at least, the percentage of total nitrogen continued to increase gradually to the time of the marked decline of the rind in September (fig. 2). Near the end of the observation period the percentage of total nitrogen began to diminish although the rind became greener.

Hesperidin.—Hesperidin was determined only in rinds of the Washington Navel variety, in which it was found to diminish during the winter to a little more than one-half the original percentage (fig. 2).

Hydrogen-ion concentration.—Hydrogen-ion concentration likewise was determined only in the rind of the Washington Navel variety. It changed very little during the entire period of observation although there was a very small but steady decrease during this period (fig. 2).

Comparison of Stem and Blossom Ends

To make a general comparison of stem and blossom ends of the rinds, all determinations belonging to each end were brought together and the average values calculated (table 2). Each average represents the albedo and flavedo for all collections, both at Pomona and at Corona. Table 2 shows a striking difference between stem and blossom ends of the rinds of both varieties, certain phases of which were reported upon by Klotz and Haas (*5*, 7). The following differences are shown in table 2 and figure 3: The rind of the stem end exceeded that of the blossom end in percentage of water, reducing sugar, total sugar, soluble solids, and soluble nitrogen;[8] the blossom-end rind exceeded the stem-end rind in invert sugar, insoluble nitrogen,[8] hydrolyzable polysaccharides, insoluble solids, crude fiber,[8] and flavedo-albedo ratio. In the Washington Navel rinds hesperidin and active acidity were likewise higher in the blossom end. Few exceptions to these differences occurred on any collection date or in either locality.

In addition, there were some very interesting seasonal variations in the difference between the two end rinds, particularly in the Valencia variety. The general summary as given in table 2 cannot disclose such seasonal trends; however, figure 3 shows how the difference between the stem and blossom ends fluctuated through the season. In Valencia oranges the difference in sugars tended to increase as the season advanced until the September 10 collection, when the maximum divergence was reached. If the graphs for sugars in Valencia oranges (fig. 3) are compared with the mean-temperature graph (fig. 1) it will be seen that the graphs are remarkably similar, indicating that the difference between the composition of the stem and blossom ends was a variable influenced by the temperature and that the higher the mean temperature the greater was the divergence. The graphs of figure 3 also show the seasonal divergence or difference between the two end rinds as regards insoluble solids, soluble solids, and hydrolyzable polysaccharides. In Valencia oranges these constituents do not exhibit a similar close parallelism with the mean-temperature graphs (as shown in figs. 1 and 2), but they agree in giving the greatest seasonal divergence for these substances as occurring at the September 10 collection. The seasonal-divergence graphs for water and nitrogens presented in figure 3 are similar to each other but do not parallel the mean-temperature graph. On analysis of the variations of the two rinds in respect to water it was found that in every case such variations were caused by the more rapid loss or the slower gain of water by the blossom end of the orange. This finding agrees with observations on the diurnal variations of water in Valencia rinds referred to on page 728, when it was noted that the blossom end showed somewhat the larger diurnal deficit.

Some emphasis has been placed here upon the seasonal variation of the difference between the two end rinds for the reason that if there is any significance in the many recorded differences between the ends of Valencia rinds in relation to storage and other spotting it should be of interest to know that the difference is strongly affected by seasonal factors.

[8] Determined only in the Valencia variety.

TABLE 2.—*A comparison of the chemical composition of orange stem-end and blossom-end rinds* [1]

VALENCIA

Portion of rind	Reducing sugar		Invert sugar		Total sugar		Soluble solids	
	Average	Range	Average	Range	Average	Range	Average	Range
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Stem end	24.59	21.91–26.39	10.30	6.95–11.96	34.89	33.11–37.24	57.57	50.98–61.20
Blossom end	15.69	11.03–19.05	10.51	7.98–14.05	26.20	24.85–28.43	50.24	47.62–52.80

Portion of rind	Insoluble solids		Hydrolyzable polysaccharides		Soluble nitrogen		Insoluble nitrogen	
	Average	Range	Average	Range	Average	Range	Average	Range
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Stem end	32.56	28.12–38.25	10.17	7.20–12.69	0.439	0.386–0.517	0.432	0.397–0.464
Blossom end	39.05	36.69–42.97	14.53	11.43–17.73	.401	.366–.451	.496	.476–.530

Portion of rind	Total nitrogen		Water		Crude fiber		Flavedo-albedo ratio	
	Average	Range	Average	Range	Average	Range	Average	Range
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Stem end	0.871	0.801–0.958	76.9	74.9–79.5	22.39	20.11–27.47	1.54	1.36–1.67
Blossom end	.897	.853–.980	68.9	67.7–69.8	24.52	23.26–26.95	1.74	1.60–1.87

WASHINGTON NAVEL

Portion of rind	Reducing sugar		Invert sugar		Total sugar		Soluble solids	
	Average	Range	Average	Range	Average	Range	Average	Range
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Stem end	32.97	25.81–37.59	5.89	4.23–6.97	38.86	31.34–43.99	62.11	57.07–65.73
Blossom end	28.01	22.24–32.27	6.05	5.29–6.93	34.06	28.12–38.24	55.25	48.92–60.18

Portion of rind	Insoluble solids		Hydrolyzable polysaccharides		Water		Flavedo-albedo ratio	
	Average	Range	Average	Range	Average	Range	Average	Range
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Stem end	37.89	34.27–42.93	12.18	10.88–13.95	75.8	73.4–78.4	0.94	0.87–1.02
Blossom end	44.75	39.82–51.08	15.02	12.97–16.75	71.7	70.2–73.2	1.15	1.10–1.23

Portion of rind	Hesperidin		Hydrogen-ion concentration	
	Average	Range	Average	range
	Percent	*Percent*	*pH*	*pH*
Stem end	2.50	0.59–3.93	5.82	5.48–6.08
Blossom end	3.38	2.67–3.78	5.56	5.27–5.74

[1] All values except water and hydrogen-ion concentration are calculated on the basis of dry weight.

Comparison of the Flavedo and Albedo

Table 3 gives a summary of all analyses arranged in such a manner as to allow comparison between the composition of the outer and the

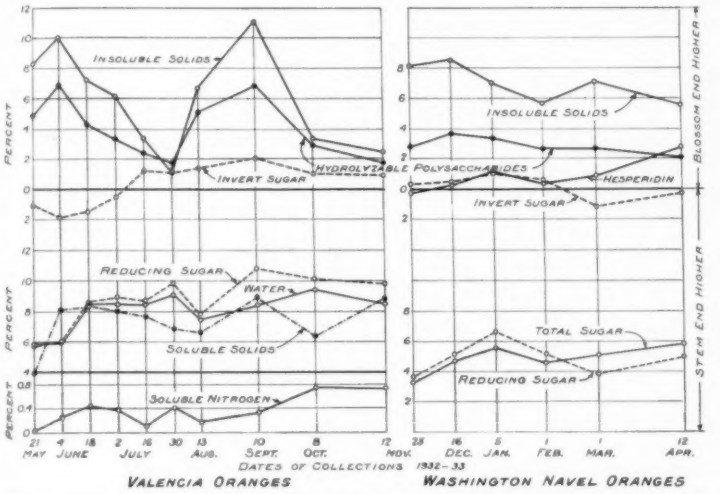

FIGURE 3.—Seasonal differences between stem and blossom ends with respect to certain constituents of the rind of Valencia and Washington Navel oranges.

inner layers of Valencia and Washington Navel orange rinds. The principal differences observed may be stated briefly as follows: In the Valencia rinds the flavedo always exceeded the albedo in weight (as indicated by the fact that the ratio always exceeded 1) and in nitrogen, but the albedo exceeded the flavedo in crude fiber. In both varieties the flavedo also had a higher percentage of reducing sugar, invert sugar, total sugar, and soluble solids; the albedo had a higher percentage of insoluble solids and hydrolyzable polysaccharides. There was no marked difference in the percentage of water. In the Washington Navel variety the albedo was higher in hesperidin and active acidity.

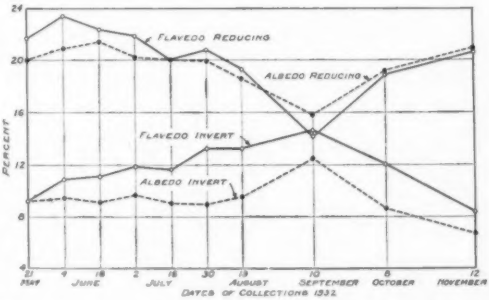

FIGURE 4.—Comparison of seasonal changes in the flavedo and albedo layer of the rind of Valencia oranges with respect to the sugar content.

In Valencias the ratio of flavedo to albedo was always greater than unity. At the beginning of the Valencia season it was 1.57, increasing to a maximum of 1.77 at the September 10 collection. The variation of this ratio seemed to be due largely to changes within the albedo.

TABLE 3.—*A comparison of the chemical composition of orange flavedo and albedo*[1]

VALENCIA

Portion of rind	Reducing sugar		Invert sugar		Total sugar		Soluble solids	
	Average	Range	Average	Range	Average	Range	Average	Range
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Flavedo	19.74	16.53–23.35	11.62	8.23–14.57	31.36	29.25–34.44	53.69	48.81–56.62
Albedo	19.01	16.16–21.76	9.12	6.69–11.44	28.13	27.87–30.92	51.44	48.22–54.21

Portion of rind	Insoluble solids		Hydrolyzable polysaccharides		Soluble nitrogen		Insoluble nitrogen	
	Average	Range	Average	Range	Average	Range	Average	Range
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Flavedo	25.80	22.93–28.43	8.12	5.14–10.98	0.503	0.462–.591	0.525	0.496–0.533
Albedo	48.54	44.00–52.99	17.04	12.78–20.07	.328	.270–.384	.408	.358–.449

Portion of rind	Total nitrogen		Water		Crude fiber		Flavedo-albedo ratio	
	Average	Range	Average	Range	Average	Range	Average	Range
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Flavedo	1.028	0.976–1.087	72.9	71.7–74.5	17.68		1.64	1.57–1.77
Albedo	.736	.665–.832	72.6	71.1–75.0	31.50			

WASHINGTON NAVEL

Portion of rind	Reducing sugar		Invert sugar		Total sugar		Soluble solids	
	Average	Range	Average	Range	Average	Range	Average	Range
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Flavedo	32.85	25.40–37.34	7.17	5.52–7.87	40.02	32.37–44.79	65.51	58.96–69.71
Albedo	28.09	22.39–32.35	4.76	4.00–5.38	32.85	26.83–37.43	51.76	47.03–56.21

Portion of rind	Insoluble solids		Hydrolyzable polysaccharides		Water		Flavedo-albedo ratio	
	Average	Range	Average	Range	Average	Range	Average	Range
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Flavedo	34.49	30.29–41.04	10.73	8.83–13.36	73.3	72.0–75.5	1.04	0.84–1.24
Albedo	48.24	43.79–52.97	16.46	15.02–17.77	74.3	72.2–76.3		

Portion of rind	Hesperidin		Hydrogen-ion concentration	
	Average	Range	Average	Range
	Percent	*Percent*	*pH*	*pH*
Flavedo	2.42	2.02–3.08	6.10	5.69–6.38
Albedo	3.47	1.32–5.57	5.25	5.07–5.40

[1] All values except water and hydrogen-ion concentration are expressed on the basis of dry weight.

The seasonal trends of sugars in the flavedo and albedo of Valencia rinds are shown in figure 4. The graphs for reducing sugar and invert sugar show the characteristic behavior of these substances. The hot-weather period of August and early September diminished the reducing sugar and increased the invert sugar decidedly. The color of the fruit remained good until after the September 10 collection, when it began to regreen rapidly. The flavedo and albedo did not differ much in their reducing-sugar content, but the former was considerably higher in invert sugar throughout the season.

Comparison of Pomona and Corona Fruit

One of the interesting facts shown by the data was the very considerable difference in the chemical composition of the rind of Valencia oranges in the two localities, Pomona and Corona. Colby (*2*) published the results of a comparative study of sugars and acid in the juice of Washington Navel oranges from different sections of California, but the writers have seen no comparative observations on the rind.

A summary of the writers' results is presented in table 4. Each value given in the table is the average of 80 or more determinations.

In Valencias the rind of the Pomona fruit had a higher content of water, reducing sugar, soluble solids, soluble nitrogen, and total nitrogen. On the other hand, the rind of the Corona Valencias was higher in invert sugar, insoluble solids, hydrolyzable polysaccharides, and the flavedo-albedo ratio. Total sugar and insoluble nitrogen show very little significant difference.

The rind of Pomona Washington Navel oranges was higher in water, hesperidin, hydrolyzable polysaccharides, and insoluble solids, but lower in reducing sugar, invert sugar, total sugar, soluble solids, hydrogen-ion concentration, and flavedo-albedo ratio. The fruit at Corona, as already stated, was somewhat more advanced in maturity on all collection dates, and therefore the fruit from the two localities was not strictly comparable.

Table 4.—*A comparison of the chemical composition of the rind of oranges grown in two localities during the season of 1932–33* [1]

VALENCIA

Locality	Reducing sugar	Invert sugar	Total sugar	Soluble solids	Insoluble solids	Hydrolyzable polysaccharides	Soluble nitrogen	Insoluble nitrogen	Total nitrogen	Water	Flavedo-albedo ratio
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Pomona, Calif.	21.12	8.88	30.60	54.18	34.98	11.24	0.479	0.451	0.930	74.9	1.55
Corona, Calif.	17.89	11.67	29.56	50.00	40.07	13.78	.360	.480	.840	70.6	1.66

WASHINGTON NAVEL

Locality	Reducing sugar	Invert sugar	Total sugar	Soluble solids	Insoluble solids	Hydrolyzable polysaccharides	Water	Flavedo-albedo ratio	Hesperidin	Hydrogen-ion concentration
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Pomona, Calif.	28.83	5.58	34.41	57.20	42.80	14.23	74.5	1.00	3.66	5.80
Corona, Calif.	32.15	6.35	38.50	60.06	39.94	12.96	73.0	1.09	2.23	5.53

[1] All values except water and hydrogen-ion concentration are calculated on the basis of dry weight.

Another factor that should be taken into consideration in this comparison is the rise in temperature that occurred at Corona during the interval between the pickings of Washington Navels at the two locations in December and January. The data were examined to find whether this difference in temperature for the few days preceding picking might have helped to widen the difference in composition of the rinds of fruit from the two localities. Graphs were constructed to show the seasonal divergence, with regard to certain substances, between the stem end and the blossom end (fig. 3) and between Pomona and Corona Washington Navel rinds taken as a whole (fig. 5). It was noted at once that the extent of divergence between the stem end and the blossom end, with regard to several substances, remained rather uniform throughout the season, but that the divergence between the Pomona and Corona rinds was considerably greater earlier in the season when the mean-temperature difference between Pomona and Corona was the greatest. If this explanation has any value, it indicates that the temperature changes at Corona on the dates stated may have affected the composition of the rinds to an extent measurable by the methods employed in this study.

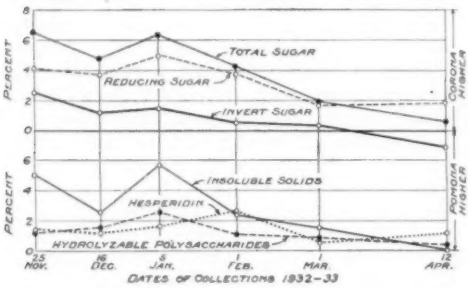

FIGURE 5.—Seasonal differences between Washington Navel oranges from Corona and Pomona with respect to certain constituents of the rind.

CHANGES UNDER STORAGE CONDITIONS

Valencia Oranges

During the November 12 collection extra quantities of fruit were picked and placed in cold storage in order to observe the changes that might take place in the rinds.

The temperatures used were 33° and 53° F. A controlled high relative humidity was maintained in the 53° room but no control of relative humidity in the 33° room. In the latter the relative humidity was always lower than in the former; in fact, enough lower to more than offset the lower vapor tension at 33°, with the result that the rinds of this lot lost noticeably more water. The period of storage was 7 weeks (Nov. 12 to Dec. 31). When the final examination was made the fruit seemed in fair condition, except for the characteristically "old" appearance and considerable shrinkage in the lot held at 33°. The difference in rate of water loss in the two storage rooms renders the interpretation of the chemical data difficult. It is clear that all such experimental storage rooms ought to be maintained at equivalent relative humidities so that the loss of water from the fruit would be approximately the same in every room.

The stem-end rinds lost water faster than the blossom-end rinds. Apparently there can be a significant difference in the behavior of the two ends in this regard, depending upon whether or not the fruit is attached to the tree. The flavedo-albedo ratio varied in storage;

the flavedo nearly always lost water more rapidly than the albedo. It is not known whether this was due to the greater exposure of the flavedo or to its smaller water-holding capacity. Some tests were made of the rate of drying when flavedo and albedo tissues were exposed in the laboratory. The flavedo lost about 26 percent of its original weight in 4½ hours, whereas the albedo lost 16 percent. Weighings at the end of 24 hours showed that the albedo tissue had continued to lose water at nearly a constant rate, making a total of 62 percent, but the rate of drying of the flavedo had so decreased after the first period that the total loss was only 44 percent. It would be interesting to test the successive changes in flavedo-albedo ratios of oranges during storage at low humidity. Soluble solids decreased in all portions of the rinds at both temperatures, and the decrease was more than twice as much at 53° as at 33° F. When the stem end and the blossom end were compared it was found that the stem-end rind had lost twice as much of its soluble solids as the blossom end.

A diminution of total sugars accounts for a large part of the change in soluble solids. Reducing sugar decreased slightly, but invert sugar decreased greatly. The latter almost disappeared when the fruit was held at 53° F. There was an apparent small increase of hydrolyzable polysaccharides in all portions of the rind in both storage rooms. However, it is doubtful whether this is a real increase, for it is decidedly contrary to the results obtained for polysaccharides during extensive companion studies of the rinds of grapefruit in storage. The changes in soluble and insoluble nitrogen in storage were so slight that no correlations were noted.

The results obtained in the determination of the hydrogen-ion concentration in the rind and in the pulp juice of the two storage lots from Pomona are presented in table 5. From these data it is evident that the rind of the fruit from the 33° F. room was more acid than that from the 53° room. This is true of all corresponding portions of the rind, the average difference being 0.26 pH. The stem-end portions of the rind were more acid than those from the blossom end, and the equatorial portions held an intermediate position. The flavedo was less acid than the albedo. The pulps showed a difference of only 0.04 pH, but in this case that held at 53° was more acid.

TABLE 5.—*Hydrogen-ion concentration determinations of different portions of Valencia oranges after being held in storage for 7 weeks at two different temperatures* [1]

Storage temperature (°F.)	Stem-end rind	Blossom-end rind	Equatorial rind	Flavedo	Albedo	Average of rind portions	Pulp juice
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
53	5.97	6.33	6.23	6.60	5.77	6.18	3.78
33	5.79	6.01	5.96	6.32	5.51	5.92	3.82
Difference	.18	.32	.27	.28	.26	.26	.04

[1] Each pH value is the average of 16 determinations. The writers are fully aware that the averaging of pH values, here and elsewhere in this paper, is mathematically unsound, but they believe that through the ranges shown, the averages of pH values as numerical entities are probably as sound biologically as the averages of the actual hydrogen-ion concentrations or the equivalent pH values, and are considerably more convenient.

WASHINGTON NAVEL ORANGES

The first storage experiment of this study was started at a time corresponding to the third field collection. The fruit came from the same trees at Pomona and Corona that were used for the regular field

collections. The fruit from each of the two localities was divided into two lots and placed in storage on January 7 and 11, respectively. The length of the storage period was 30 days. One lot from each locality was held at 52° F. with an average relative humidity of 89 percent, and the other lot was held at 32° with an average relative humidity of 76 percent. At the end of this period the fruit was free from rind blemishes at both temperatures. Chemical analyses of the rinds of these and the following lots were carried through exactly as described for the regular field collections.

The time of collection for the second storage experiment corresponded with that of the fifth field collection, March 1 to 4. Lots from each locality were stored for 46 days at 52°, 42°, and 32° F. with average relative humidities of 89 percent (range 85–93 percent), 85 percent (range 83–86 percent), and 85 percent (range 83–87 percent), respectively. At the end of this time none of the fruit showed any rind blemishes except the lot from Corona held at 32°. In this lot 72 percent of the fruit showed a brown stain somewhat resembling common apple scald.

The third storage experiment was carried out with Corona fruit only, and the time of picking corresponded with that of the sixth field collection, April 13. The temperatures and relative humidities used were the same as for the second storage experiment. The length of the storage period was 33 days, after which the rinds were found to be free from storage blemishes except in the lot held at 32° F. All of this fruit showed various amounts of brown stain.

The fourth storage experiment was made with Pomona fruit only, and the time of picking corresponded with that of the seventh [9] field collection, May 25. The temperatures and relative humidities were again the same as those used in the second storage experiment. The length of storage was 41 days. At the end of this period there were no storage blemishes on the rinds except in fruit of the lot held at 32° F., all of which showed brown stain. The fruit had been picked after the regular commercial season, and none of it was very firm when placed in storage; at the time of removal from storage it was somewhat soft and not fresh looking. This last statement applies nearly as well to the Corona fruit used in the third storage experiment. This fruit had been picked nearly 6 weeks before the Pomona fruit of the fourth storage experiment, yet the fruit of both locations seemed to be at about the same stage of maturity when placed in storage.

EFFECTS OF TEMPERATURE

On Rind as a Whole

External observations of the oranges used in the simple storage experiments described above pointed to two tentative conclusions, namely, (1) the fruit was most susceptible to brown stain at 32° F., and (2) it was also more susceptible during the latter part of the season.

The effects of storage temperatures on the chemical composition of Washington Navel orange rinds are shown in table 6. The values in this table are from the data from the second, third, and fourth storage experiments. Data obtained from the first storage experiment are not included because there was no storage at 42° F.

[9] Since, as already stated, the 7th field collection was made only at Pomona, to serve as a control for the storage experiment, the analytical data from it were not included in the presentation of changes in the field but were used for the average percentages at beginning of storage given in table 5.

It is evident from table 6 that certain substances in the rind gave definite responses to the temperatures used. Invert sugar showed only a small decrease at 32° F. but a very large one at 52°. Reducing sugar showed a smaller decrease, which was progressively reduced as the temperature of storage was lowered. The striking alteration in invert sugar had little effect on the general trend of total sugar because invert sugar comprised scarcely more than one-tenth the amount of reducing sugar. Total sugar, therefore, behaved much like reducing sugar.

TABLE 6.—*Effects of temperature on the composition of the rind of the Washington Navel orange in storage* [1]

Storage conditions	Reducing sugar		Invert sugar		Total sugar		Soluble solids	
	Content	Loss	Content	Loss	Content	Loss [2]	Content	Loss
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Before storage	34.67		5.94		40.61		63.0	
After 46 days at—								
52° F.	33.43	3.58	3.14	47.1	36.57	9.9	60.4	4.2
42° F.	34.01	1.91	3.66	38.4	37.67	7.2	60.7	3.7
32° F.	34.25	1.21	5.46	8.1	39.71	2.2	62.8	.3

Storage conditions	Insoluble solids		Hydrolyzable polysaccharides		Hesperidin		Hydrogen-ion concentration	
	Content	Gain	Content	Loss (−) or gain(+)	Content	Gain	pH	Difference in pH
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*		
Before storage	37.0		11.89		2.12		5.94	
After 46 days at—								
52° F.	39.6	7.1	12.57	+5.72	3.14	48.1	5.47	0.47
42° F.	39.3	6.2	12.41	+2.29	3.04	43.4	5.52	.42
32° F.	37.2	.54	11.79	−.93	2.65	25.0	5.83	.11

[1] All percentages are calculated on the basis of dry weight.
[2] The percentage of loss or gain is calculated on the basis of the percentage present at the beginning of the storage period.

The recorded increase of hesperidin in storage is very interesting. At 52° F. hesperidin increased to one and one-half times the quantity present at the beginning of storage, and even at 32° it increased to one and a quarter times that originally present. That this is an actual increase in the quantity of hesperidin seems certain, for if the loss of sugar and other solids is taken into account, there is still a clear margin showing increase of this substance at all temperatures used. This synthesis of hesperidin in storage seems to be correlated with the rate of respiration, and its accumulation with the lack of translocation. Hesperidin was found to decrease steadily through the season while the fruit was on the tree. This fact suggests a number of possibilities, of which one is that while translocation of this rather stable glucoside (or group of glucosides) can continue while the fruit is on the tree, there may be a diminution of the rate of respiration as the orange matures. Thus one might account for the seasonal decrease. It can scarcely be assumed that the synthesis of hesperidin ceases completely at any time in the field.

Soluble solids showed the greatest loss at 52° F. and the least at 32°. The insoluble solids consequently had an apparently opposite trend.

Hydrolyzable polysaccharides apparently increased in storage at 52° and 42° F., but if the loss of other substances is taken into account it is found that they remained practically stationary, or possibly decreased slightly at all temperatures.

When the rinds were considered as a whole it was found that the flavedo-albedo ratio remained practically unchanged in storage. However, if stem and blossom ends were compared separately it was found that in the stem end the ratio made an average increase of 0.04 at 32° F., a decrease of 0.03 at 42°, and a decrease of 0.04 at 52°. In the blossom end the changes were almost exactly reversed, the changes in value of the ratios being a decrease of 0.04 at 32°, a decrease of 0.03 at 42°, and an increase of 0.06 at 52°.

Under all conditions of storage there was an increase of active acidity in all portions of the Washington Navel orange rind (table 7). The greatest increase was at 52° F. and the least at 32°. This effect of temperature is quite the reverse of that recorded for the Valencia orange rind, which will be referred to again in the comparison of the two orange varieties.

On Portions of Rind

In all the preceding discussion of the effects of different temperatures on the rind of Washington Navel oranges in storage, the rind has been considered as a whole, except for a brief mention of the flavedo-albedo ratios in the rind of the stem and blossom ends.

A detailed account of the relative behavior of the stem and blossom ends, of the flavedo and albedo, or of fruit from the two localities, would seem undesirable in a summarized report of this kind. Nevertheless, it may be stated that the different portions of the rind did often respond to storage conditions somewhat differently, although these responses differed in quantity only.

Table 7.—*Hydrogen-ion concentration of different portions of the rind of Washington Navel oranges held in storage 46 days at different temperatures*

Storage temperature (° F.)	Stem end	Blossom end	Flavedo	Albedo	Average [1]
	pH	*pH*	*pH*	*pH*	*pH*
Before storage (control)	6.15	5.74	6.39	5.49	5.94
52°	5.69	5.25	5.74	5.20	5.47
42°	5.75	5.29	5.79	5.24	5.52
32°	6.03	5.63	6.24	5.42	5.83
Average [1]	5.82	5.39	5.92	5.29	5.61

[1] See footnote 3 to table 1.

The stem end showed greater changes than the blossom end in all the substances under observation except active acidity. The immediate explanation of this was found to involve the relative distribution of the albedo tissue, which is more responsive to everything except acidity. The albedo is more abundant in the stem end (the flavedo-albedo ratio was less than unity here), and the reverse is true of the blossom end. Why the flavedo tissue of the Washington Navel

orange in storage should show greater change in active acidity than the albedo is not clear, but the condition appears to be quite different from that found in studies on the rind of grapefruit, which will be reported separately, where the albedo showed greater responses in acidity. This greater activity in acidity responses of the flavedo of the Washington Navel orange rind might possibly have some relation to the surface brown stains noted in storage, in contrast to the deeper seated pittings noted in the grapefruit rind where the albedo shows the greater acidity response.

COMPARISON OF RINDS OF WASHINGTON NAVEL AND VALENCIA ORANGES

When any attempt is made to compare the rinds of the Washington Navel and Valencia oranges in southern California, it must be kept in mind that almost none of the chemical differences between them can be attributed definitely to the fact that the oranges are of different varieties. It has already been mentioned that the two varieties are harvested at different seasons of the year. In southern California the Washington Navel orange season begins in the late fall and ends in the spring, whereas the Valencia orange season begins in the spring and extends through the summer. The average weather conditions affecting the two varieties at harvest time differ as widely as is possible for two intervals of such length in the district; for example, the mean temperature for the entire period during which the Washington Navel orange material was collected was 52.1° F., whereas the corresponding mean for the period of Valencia collections was 67.3°. During the season of the Washington Navel orange there were many minima of 32° or below, and in the Valencia season there were several maxima of 100° or above. The opinion has been advanced that the temperature and other environmental conditions immediately preceding the picking of the fruit are capable of producing considerable and rapid chemical changes within the rind tissue. If this be true it seems probable that recorded differences between Washington Navel and Valencia orange rinds will have been influenced by the seasonal differences in the weather affecting each variety immediately preceding and during picking. In other words, if the environmental conditions present at the harvest time of the two varieties were reversed, then the chemical situation in the rinds might also be reversed, or at least considerably altered. It is to be expected that such wide differences in temperature and other climatic factors at the time of harvest might induce a difference in the rinds of the two varieties that would be reflected in their behavior in storage and transit. With the foregoing caution against accepting differences between rinds of the Washington Navel and Valencia oranges as altogether varietal, the following comparisons are presented.

In the Field

A general summarized comparison, presented in table 8, shows the average of all chemical determinations for both varieties as they came from the grove. Each value for the Washington Navel rind in the table is the average of 96 determinations, and each value for the Valencia rind is the average of 160 determinations.

TABLE 8.—*A comparison of the chemical composition of the rind of Washington Navel and Valencia oranges before storage* [1]

Variety	Water	Reducing sugar	Invert sugar	Total sugar	Soluble solids	Hydrolyzable polysaccharides	Reducing-invert sugar ratio	Flavedo-albedo ratio
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Washington Navel [2]	73.8	30.49	5.97	36.46	58.68	13.60	5.11	1.04
Valencia [3]	72.0	19.38	10.37	29.75	53.91	12.58	1.87	1.64

[1] All values except those in the first and the last 2 columns are calculated on the basis of dry weight.
[2] Average of 96 determinations.
[3] Average of 160 determinations.

The Washington Navel rind showed a slightly higherw ater content than the Valencia rind. This might easily be the effect of the higher temperatures and greater evaporating power of the air during the Valencia season. As to seasonal trend, the Washington Navel rind showed a small decrease and the Valencia rind a small increase as the season advanced.

Reducing sugar was more than one and one-half times as abundant in the Washington Navel rind. On the other hand, invert sugar was nearly twice as high in the Valencia rind. Total sugar, however, in the Washington Navel rind was nearly one-fourth more than that in the Valencia rind. The difference in value of the reducing-invert sugar ratio was surprising. In the Washington Navel rind this ratio was 2.7 times as great as in the Valencia rind.

During the season total sugars remained practically constant in the Valencia rind but increased steadily in the Washington Navel rind. The graph (fig. 2) showing seasonal variations in reducing sugar for the Valencia rind paralleled the temperature graph; in the Washington Navel rind reducing sugar increased steadily. The seasonal invert-sugar graph for Valencias was in general the inverse of the reducing sugar graph, but in the Washington Navel rind this was not true.

Soluble solids and hydrolyzable polysaccharides were more abundant in the Washington Navel rind. Soluble solids increased steadily throughout the season in this variety but increased or diminished almost inversely with the temperature in the Valencia rind.

Hydrogen-ion concentration was higher in the stem-end than in the blossom-end rind of Valencias, but in the Washington Navel rind the hydrogen-ion concentration was lower in the stem end. The hydrogen-ion concentration of the rinds as a whole was higher in the Washington Navel orange.

The average flavedo-albedo ratios of Washington Navel and Valencia orange rinds were 1.04 and 1.64, respectively. The smaller ratio for the Washington Navel was due immediately to its considerably heavier albedo. The flavedo also was somewhat heavier than that of the Valencia orange, but this difference was more than offset by the relatively heavier albedo.

It was noted that at Pomona the Valencia rind had more reducing sugar and soluble solids and less hydrolyzable polysaccharides than at Corona. In the Washington Navel rind conditions were exactly the reverse.

In Storage

For a comparison of the relative behavior of the Washington Navel and Valencia orange rinds in storage, the data from only two temperatures, 52° and 32° F., are available, since no Valencia oranges were stored at 42°.

Table 9 gives some average changes in the composition of the rinds of the two varieties in storage. In general, the Valencia rind made greater responses to storage conditions than did the Washington Navel orange rinds. It seems possible that the condition of the Valencias in the field, influenced as they were by hot, dry weather, might have brought about the greater changes that occurred in this variety in storage. For most collections, the temperature of the Washington Navel oranges was actually raised when taken from the field to storage at 52° F. Changes at this temperature were greater in both varieties, except in the hydrogen-ion concentration. In the Valencia rind the apparent increase of reducing sugar at 52° was probably due to the very abundant invert sugar, which for a time was hydrolyzed at a rate exceeding that at which it was consumed by respiration.

Table 9.—*Comparison of the composition of Washington Navel and Valencia orange rinds before and after storage at different temperatures* [1]

Variety and storage conditions	Reducing sugar	Invert sugar	Total sugar	Hydrolyzable polysaccharides	Soluble solids
Washington Navel:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Before storage	34.67	5.94	40.61	11.89	63.05
After 42 days at—					
52° F	33.43	3.14	36.57	12.57	60.44
32° F	34.25	5.46	39.71	11.79	62.78
Valencia:					
Before storage	21.69	7.47	29.16	10.12	61.20
After 48 days at—					
53° F	22.33	2.54	24.87	13.77	54.85
33° F	19.75	6.38	26.13	12.20	52.63

[1] All values are calculated on the basis of dry weight.

As mentioned in the presentation of the storage data for the Washington Navel orange rind, the changes in hydrogen-ion concentration in storage were strikingly different in the two varieties. The Valencia rind developed a greater acidity at 33° than at 53° F., the pH averages of which were 5.92 and 6.18, respectively, but the Washington Navel rind consistently showed the reverse effect, the average pH values being 5.83 and 5.47 at 32° and 52°, respectively.

DISCUSSION

While it is freely admitted that the results reported cannot at present be definitely linked with pitting and spotting in storage, there are some general aspects of the findings that are worthy of notice.

Since orange rinds were found to respond sometimes very strikingly to changes in the environment, it is suggested that these responses probably relate closely to the behavior of the rinds after picking. Even a moderate understanding of this relationship might modify picking practices considerably, especially for fruit destined for

export or storage. At present the more vigilant packing-house managers do not wish Valencias picked during periods of unusually hot weather and insist that they shall not be picked except during a few hours in the early morning. So meteorology may come to play as important a role in the picking schedule as it now does in spraying and frost control.

It has been generally assumed that the susceptibility to spotting of fruit coming from the grove depends somewhat, at least, upon the district in which the fruit is grown, the season, stage of maturity, and general cultural treatment received in the grove. However, some of the responses of rinds observed in this work seem to indicate that a given orange on different days may present a significantly different aspect as regards its possible resistance to subsequent conditions in storage and transportation that favor pitting and spotting; that is to say, the susceptibility of an orange to spotting in storage is probably not merely "high" in very early and very late stages of maturity, but it may vary from week to week, being sometimes high and sometimes low, depending upon the environmental factors of the few days preceding the picking of the fruit.

A study of the relation of storage and transit conditions to pitting and spotting is still in progress.

SUMMARY AND CONCLUSIONS

Results are presented of a study of changes taking place in the rind of oranges in the field and in storage during the season of 1932–33 at Pomona and Corona, Calif.

Seasonal changes in the composition of the rind as a whole were as follows:

(1) In Valencia oranges there was a slight seasonal upward trend in water content, but in Washington Navels the reverse was true, while in both varieties there were fluctuations due to immediate environmental causes.

(2) In Valencias the flavedo-albedo ratio was always greater than unity, increasing temporarily in hot weather; in Washington Navels the ratio varied on either side of unity and likewise fluctuated with weather conditions.

(3) In Valencias the soluble solids varied inversely with the mean temperature, but in Washington Navels there was a general relative increase throughout the season.

(4) In Valencias there was practically no change in total sugar during the season but invert sugar increased as reducing sugar decreased. In Washington Navels, on the other hand, total sugar increased throughout the season, primarily because of the steady increase of reducing sugar; invert sugar fluctuated with immediate environmental factors.

(5) In Valencias the hydrolyzable polysaccharides varied with the mean temperature, but in Washington Navels they decreased as the season advanced.

(6) Nitrogen content was studied only in Valencia oranges. Only a slight change was observed during the season, with some fluctuations in the proportion of the soluble and insoluble fractions.

(7) Hesperidin and hydrogen-ion concentration were determined only in the rinds of Washington Navel oranges. Hesperidin decreased

steadily during the season to about one-half the amount present early in the season. Hydrogen-ion concentration made a small but definite decrease during the season.

A comparison of the stem and blossom ends showed a definite difference in composition during the season. In Valencias the stem end was higher in water, reducing sugar, soluble nitrogen, and soluble solids, while the blossom end had a greater amount of total sugar per 100 ml of solute than the stem end because of the higher water content of the stem end. These differences varied through the season, being greatest during the hottest weather. In Washington Navels the stem end was likewise higher in water, reducing sugar, total sugar, and soluble solids and the blossom end was higher in invert sugar, hydrolyzable polysaccharides, insoluble solids, hesperidin, hydrogen-ion concentration, and flavedo-albedo ratio.

A comparison of the flavedo and albedo showed that in the Valencia the flavedo always weighed more than the albedo and that while there was some variation in the ratio in the Washington Navel it averaged more than unity. In the Valencia the flavedo contained a higher percentage of invert sugar, total sugar, soluble solids, and nitrogen. In the Washington Navel the flavedo was likewise higher in percentage of reducing sugar, invert sugar, and soluble solids. The albedo was higher in water, insoluble solids, and hydrolyzable polysaccharides. In the Washington Navel the albedo was higher in hesperidin and hydrogen-ion concentration. In Valencia rinds the flavedo-albedo ratio was highest during the hottest weather.

When oranges from Pomona were compared with those from Corona the rinds were found to differ in several respects. In Valencias the Pomona fruit was higher in water, reducing sugar, soluble solids, and total nitrogen, while in the Washington Navel it was higher in water, hesperidin, hydrolyzable polysaccharides, and insoluble solids. The Corona Valencias were higher in invert sugar, insoluble solids, hydrolyzable polysaccharides, and flavedo-albedo ratio, while the Corona Washington Navels were higher in both reducing and invert sugars, soluble solids, hydrogen-ion concentration, and flavedo-albedo ratio.

Storage experiments were conducted to determine the response at different temperatures. With Valencias storage experiments at 33° and 53° F. for 7 weeks gave the following results: (1) The stem-end rind lost water more rapidly than did the blossom-end rind; (2) soluble solids always decreased more rapidly at 53° than at 33°; (3) the stem-end rind lost soluble solids twice as rapidly as did the blossom-end rind; (4) reducing sugar changed very little and invert sugar almost disappeared at 53°; (5) rind from fruit stored at 33° had a higher hydrogen-ion concentration than that stored at 53°; and (6) the stem-end rind of stored fruit had a higher hydrogen-ion concentration than did the blossom-end rind. There was practically no difference in the active acidity of the pulp juice of the two lots.

In the storage experiments on Washington Navel oranges the temperatures employed were 52°, 42°, and 32° F. The responses to storage conditions were as follows: (1) The stem-end rind always showed greater responses to storage conditions than the blossom-end rind except in acidity; (2) in all portions of the rind and under all storage conditions acidity increased, the increase being greatest at 52° and least at 32°; (3) invert sugar decreased slightly at 32° but

greatly at 42° and 52°; (4) reducing sugar changed much less than invert sugar but in the same direction; (5) hesperidin showed very definite increases in storage, the increase being relatively greatest at 52°; (6) soluble solids decreased most at 52°; (7) hydrolyzable polysaccharides changed very slightly at any temperature; (8) the flavedo-albedo ratio remained unchanged in the rind as a whole, but there was a small increase of the ratio in the stem end that was exactly offset by a decrease in the blossom end.

In considering the results on Washington Navel and Valencia oranges it must be remembered that in southern California the Washington Navel oranges are harvested in winter and the Valencias in summer, which makes it difficult, in a comparison of the two varieties as grown there, to distinguish between the differences which are varietal and those which are caused by environment.

The results of storage experiments with Washington Navel oranges indicate that late-picked fruit is most susceptible to brown stain and that susceptibility is increased by storage at 32° as compared with 42° and 52° F. With respect to pitting and spotting the results are not so clear-cut but seem to show that susceptibility to these disorders varies with the environmental factors of the last few days preceding the picking of the fruit.

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FIELD AND STORAGE STUDIES ON CHANGES IN THE COMPOSITION OF THE RIND OF THE MARSH GRAPEFRUIT IN CALIFORNIA[1]

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INTRODUCTION

This paper presents the results of two closely related studies. The first section deals with the rind of the Marsh grapefruit (*Citrus grandis* (L.) Osbeck) in the field during the harvest season, and the second section deals with the rind of comparable fruit held in storage at different temperatures or under other experimental conditions.

As in the major portion of the studies on Valencia and Washington Navel orange rinds previously reported (*13*),[2] the object of the first study was to determine, as closely as the methods employed would permit, the normal seasonal changes of grapefruit rind in the field. The experiments were so designed that the information obtained might be used as the basis for a rather extensive investigation of the behavior of grapefruit rind in storage. The results of this latter study are reported in the second part of this paper.

REVIEW OF LITERATURE

The literature of previous work on the grapefruit rind is similar to that noted in connection with the orange (*13*) rind in that the attention of investigators has been directed mainly to the essential oils from a pharmaceutical or related standpoint, and when other substances have been taken into account the normal seasonal trends have not usually been considered.

One of the most extensive series of observations on seasonal changes in grapefruit is that reported by Lopez-Dominguez (*17*, *18*) dealing with Duncan and Marsh varieties. He noted changes in composition of the fruit as affected by maturity, soil type, and rainfall. The more significant portion of his report concerns the changes in the pulp and juice, but information is also given on seasonal variations in thickness of the rind. In both varieties the rind decreased in thickness with maturity; in the Marsh the rate of decrease was checked when the solids-acid ratio reached 7. Hawkins (*14*) in his study of grapefruit ripening noted that the percentage of rind decreased throughout the season and that this was paralleled by a decrease in thickness. The changes taking place in the pectic constituents of the albedo of Florida grapefruit rind have been studied by Gaddum (*8*). Caldwell (*2*) has followed the changes in hydrogen-ion concentration and chemical composition in entire Florida grapefruit, beginning with extremely immature stages and including samples taken at weekly intervals until the beginning of the commercial harvest period.

[1] Received for publication Aug. 30, 1935; issued June 1936.
[2] Reference is made by number (italic) to Literature Cited, p. 786.

As regards seasonal development of the fruit his study ends about where the present one begins, and does not deal at all with the rind alone.

Other reports dealing with the analysis of the grapefruit rind do not refer to successive changes but present observations either on the distribution of certain substances (other than essential oils) in the rind or on the differences in composition between different portions of the rind. For example, Willimott and Wokes (*22, 23*) found that in grapefruit peroxidases are concentrated in the flavedo, although these substances are fairly widely distributed in the rinds of orange and lemon. These authors also studied the distribution of vitamins in grapefruit rind. Klotz and Haas (*16*) observed that the stem end of grapefruit had a greater permeability than the blossom end.

FIELD STUDIES

MATERIALS AND METHODS

Two field studies were made. One was carried out in the district that includes Fontana and Corona, Calif.; and the other was carried out in a single locality near Oasis, Calif., in the Coachella Valley.

The field portion of the first study extended through 171 days, beginning March 19 and ending September 6, 1933. This spring and summer period coextends in general with the regular harvest of grapefruit in the western part of southern California.

The second study began November 6, 1933, and ended April 9, 1934, a period of 154 days. This winter and spring period also coextends in general with the commercial harvest of grapefruit in the Imperial and Coachella Valleys.

These observations allow a comparison of rinds of the same variety of grapefruit grown in two districts so distinctly different in climate that in one the harvest time is in the summer and in the other in the winter. This difference in harvest seasons is practically the same as that encountered in regard to the Valencia and Washington Navel oranges (*13*), but in the latter case the two varieties were grown in the same climatic district.

The mean temperatures at Fontana or Corona and at Oasis during the periods of observation differed less than might have been expected, but the climatic environment differed greatly. In the Coachella Valley the harvest season follows an extremely hot and dry summer, while in the Fontana-Corona district it follows a relatively cool period.

All fruit used in this study was obtained from trees carefully selected at the beginning of the season, five each at Fontana and Corona, and six at Oasis. The soil types in which the experimental trees were growing at Fontana, Corona, and Oasis were Hanford gravelly sand, Yolo gravelly loam, and Superstition sand, respectively.

Collections were made at intervals of approximately 6 weeks. At Corona and Fontana 10 fruits were picked from each of the 5 selected trees and at Oasis 8 fruits were picked from each of the 6 trees. The fruit was brought into the laboratory, where the rinds were removed and separated into four portions as follows: (1) Stem-end flavedo, (2) stem-end albedo, (3) blossom-end flavedo, and (4) blossom-end albedo.

The procedure followed in the determination of hydrogen-ion concentration was the same as that described in the report on Washington Navel orange rind (*13*), except that when this determination was made in the pulp juice the tissue was not frozen before pressing and the juice was diluted to four times its original volume before the analysis. The samples of pulp juice were also titrated for total acidity. The titrations were made in duplicate, using 50 ml of juice for each and diluting with 100 ml of distilled water; normal sodium hydroxide was used for the neutralization, with phenolphthalein as indicator.

Naringin was determined by the method used for hesperidin in the Washington Navel orange rind (*13*), except that the weight of the derivative sugar was multiplied by the factor 1.60 instead of 2.17 in order to obtain the weight of anhydrous naringin. The procedures in all other analyses were the same as those described in the report on orange rinds (*13*).

The sugars were calculated as follows: (1) "Reducing sugar", as glucose, from the copper equivalent to the permanganate titration before inversion; (2) "invert sugar", as invert sugar, from the copper equivalent to the permanganate titration after inversion minus the permanganate titration before inversion; and (3) "total sugar", as the sum of "reducing sugar" plus "invert sugar."

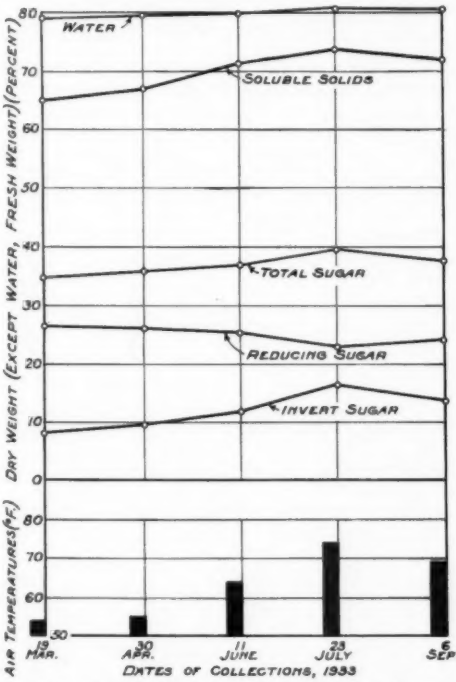


FIGURE 1.—Seasonal changes in mean air temperature (means for 5-day periods preceding collections) and in the composition of the rind of Marsh grapefruit from Corona and Fontana, Calif., with respect to water, soluble solids, and reducing, invert, and total sugars.

Since the Fontana and Corona locations are so similar climatically, the data from them are handled together in all comparisons with the data from Oasis.

EXPERIMENTAL RESULTS

Seasonal Changes

at Fontana and Corona

The seasonal changes in the composition of the grapefruit rind at Fontana and Corona are summarized in table 1 and figures 1 and 2. Each percentage value given in this table is the average of 16 determinations.

The water content of the rind did not change greatly, although there seemed to be a very slight upward trend through the season. This must mean that the water-holding power of the rind tissue increased also, otherwise there would have been a loss of water owing to the fact that the season was growing warmer and less humid until the time of the fourth collection (July 23).

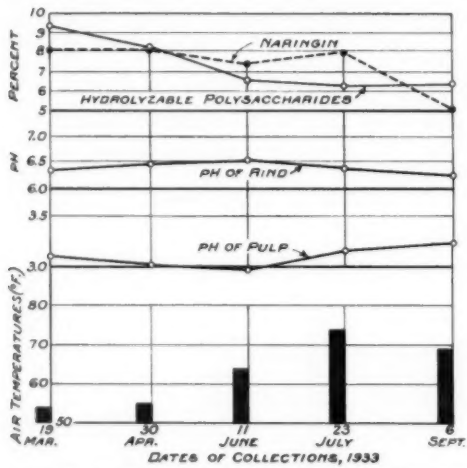


FIGURE 2.—Seasonal changes in mean air temperature (means for 5-day periods preceding collections) and in the composition of the rind of Marsh grapefruit from Corona and Fontana, Calif., with respect to naringin, hydrolyzable polysaccharides, and hydrogen-ion concentration. The pH of the pulp is also shown.

Reducing sugar decreased to a minimum at the fourth collection, whereas invert sugar increased to a maximum at that time (fig. 1). If the graphs representing the seasonal changes of these sugars are compared with the block graphs showing the mean air temperatures at Fontana and Corona, it will be noted that the reducing sugar changed somewhat inversely to and the invert sugar parallel to the temperature changes.

Total sugar behaved as did invert sugar, which dominated the total, not in percentage but by its greater seasonal changes.

TABLE 1.—*Seasonal changes in the chemical composition of the rind of Marsh grapefruit grown at Corona and Fontana and at Oasis, Calif., during the season of 1933–34*[1]

CORONA AND FONTANA, AVERAGES OF 16 DETERMINATIONS

Date of collection	Water	Reducing sugar	Invert sugar	Total sugar	Naringin	Hydrolyzable polysaccharides	Soluble solids	Insoluble solids	Hydrogen-ion concentration of rind	Flavedo-albedo ratio	Hydrogen-ion concentration of pulp juice	Milliliter N/1 acid in 10 ml pulp juice
1933	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*pH*	*Percent*	*pH*	
Mar. 19	78.9	26.55	8.10	34.65	8.07	9.40	65.1	34.9	6.31	0.74	3.09	3.74
Apr. 30	79.4	26.09	9.53	35.62	8.07	8.33	67.1	32.9	6.45	.75	3.01	3.66
June 11	79.7	25.12	11.67	36.79	7.40	6.60	71.1	28.9	6.53	.77	2.97	3.47
July 23	80.7	23.06	16.30	39.36	7.97	6.23	73.7	26.3	6.36	.78	3.16	2.99
Sept. 6	80.6	23.81	13.63	37.44	5.07	6.40	72.2	27.8	6.24	.71	3.22	2.62

OASIS, AVERAGES OF 8 DETERMINATIONS

1933												
Nov. 6	74.1	14.23	7.56	21.79	9.21	14.88	52.6	47.4	5.74	0.79	3.28	2.69
Dec. 11	74.4	18.55	9.24	27.79	7.21	12.92	58.1	41.9	6.01	.88	3.20	2.47
1934												
Jan. 15	75.1	27.04	7.69	34.73	4.93	10.59	64.7	35.3	6.26	.83	3.25	2.50
Mar. 1	80.1	22.69	9.95	32.64	5.76	10.85	63.1	36.9	6.71	.82	3.22	2.61
Apr. 9	80.3	20.38	14.00	34.38	5.26	8.87	66.7	33.3	6.46	.96	3.47	2.11

[1] All values except those in the second and the last 4 columns are calculated on the basis of dry weight.

Soluble solids increased notably until the fourth collection, when they reached a maximum. Their graph (fig. 1) was similar to that of total sugar, which accounted for slightly more than half of the soluble solids.

Naringin (fig. 2) fluctuated considerably in various samples, but on the whole decreased during the season.

Hydrolyzable polysaccharides (fig. 2) diminished slowly throughout the season, or at least became relatively less.

Hydrogen-ion concentration in the rind changed very slightly, but there appeared to be an increase until the third collection (June 11), after which there was a small decrease, so that the pH was nearly the same at the time of the first and the last collections (fig. 2).

The average hydrogen-ion concentration of the pulp juice did not parallel that of the rind. The pH graph for pulp juice is presented in figure 2, and the titration values for the same juice samples are shown in table 1.

The flavedo-albedo ratio rose slowly until the July 23 collection and then decreased to a value less than that at the beginning of the season.

AT OASIS

A summary of the seasonal changes in the rind of the Oasis grapefruit is presented in table 1 and in figures 3 and 4. Each percentage value is the average of eight determinations.

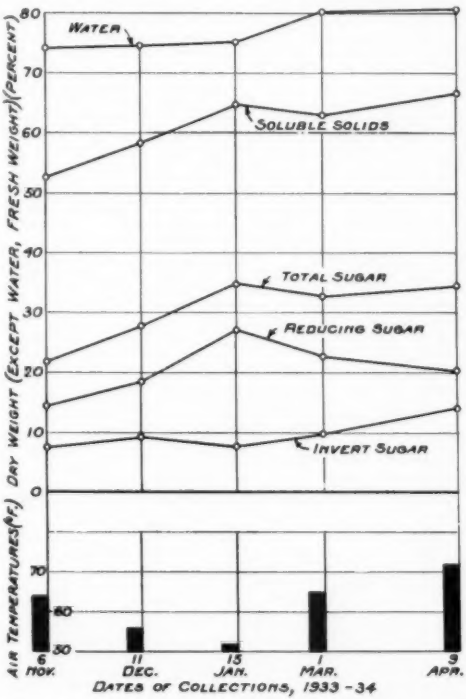


FIGURE 3.—Seasonal changes in mean air temperature (means for 5-day periods preceding collections) and in the composition of the rind of Marsh grapefruit from Oasis, Calif., with respect to water, soluble solids, and reducing, invert, and total sugars.

Water increased slightly through the season, regardless of the fact that the weather was growing warmer and drier. The average relative humidity in the Coachella Valley for March and April 1934 was about 30 percent.

Soluble solids and total sugar (fig. 3) increased rather steadily through the season. The behavior of the total sugar was largely that of the reducing sugar until invert sugar increased greatly toward the end of the season.

Reducing sugar increased until January 15 and then decreased to April 9, whereas invert sugar increased slightly to December 11, then

decreased to January 15, and increased during the rest of the season. The seasonal graphs in figure 3 show that these sugars when compared with graphs for mean air temperature behaved very much as did the same sugars in the rind of the Fontana-Corona grapefruit (fig. 1) when their graphs were compared with block graphs for mean air temperature.

The hydrolyzable polysaccharides (fig. 4) showed a relative decrease through the season.

Naringin showed a general decrease, but there were fluctuations in naringin content resembling those noted in the rinds at Fontana and Corona. It seems very probable that naringin is an indirect by-product of respiration. If so, there should be a tendency for naringin content to parallel the rate of respiration. Phloridzin, a closely related glucoside, has been found to behave so in apple shoots (*11*, *12*). If the naringin situation is somewhat comparable, one may expect a general seasonal decrease of naringin while the fruit is attached to the tree and the rate of respiration becomes slower and translocation or destruction of naringin continues. But local conditions which speed up respiration might temporarily offset the general seasonal tendency of the glucoside to diminish. The naringin graphs (figs. 2 and 4) show fluctuations which may be tentatively explained in this manner by noting the larger variations in the air temperature.

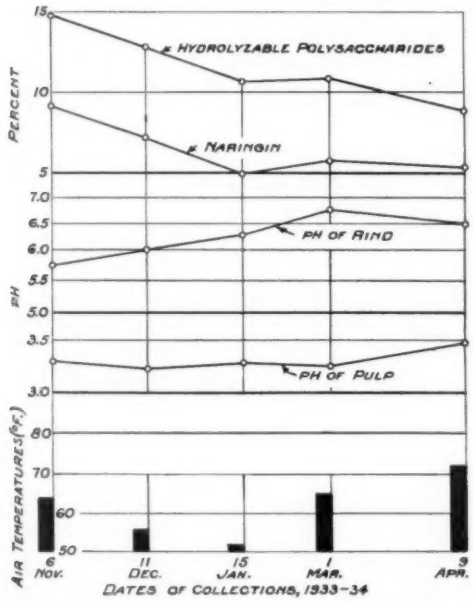


FIGURE 4.—Seasonal changes in mean air temperature (means for 5-day periods preceding collections) and in the composition of the rind of Marsh grapefruit from Oasis, Calif., with respect to naringin, hydrolyzable polysaccharides, and hydrogen-ion concentration. The pH of the pulp is also shown.

The decrease in the average hydrogen-ion concentration of the Oasis grapefruit rind was equivalent to about one pH unit during the season. The average hydrogen-ion concentration in the pulp juice also decreased, but the change was very slight.

The flavedo-albedo ratio changed as much as 21 percent, but the changes were rather irregular.

It is interesting to note that the seasonal changes in the rind of grapefruit just recorded agree in general with the findings of investigators who have studied seasonal changes in the pulp or the juice of grapefruit. In Florida, Collison (*5*) found that both reducing and

invert sugars increased in the juice while the grapefruit was attached to the tree, but that the changes were not so marked as in oranges. Invert sugar began to break down toward the end of the season. Acid also decreased gradually from the beginning of the season. Lopez-Dominguez (*17*) made the following observations on seasonal changes in the juice of grapefruit grown in Puerto Rico: Total solids remained fairly constant, acid decreased steadily; total sugar increased gradually to a solids-acid ratio of 7 and then fluctuated; and invert sugar increased throughout the season. These results are in general agreement with the findings of Hawkins (*14*) for Florida grapefruit.

Comparison of Stem and Blossom Ends at Fontana and Corona

The data presented in table 2 have been arranged for comparing the stem-end and blossom-end portions of the rind of grapefruit from Fontana and Corona. The differences in composition recorded for these rind portions were surprisingly small in view of the very much larger corresponding differences noted in the studies on Valencia and Washington Navel orange rinds (*13*).

The stem-end rind had more water, reducing sugar, invert sugar, and soluble solids than the blossom end. The latter was higher in hydrolyzable polysaccharides and hydrogen-ion concentration, but, as mentioned above, none of the differences is large, and perhaps some are of questionable significance in spite of the large number of analyses involved.

TABLE 2.—*A comparison of the chemical composition of Marsh grapefruit stem-end and blossom-end rinds at Corona and Fontana and at Oasis, Calif., during the season of 1933–34* [1]

CORONA AND FONTANA, 1933

Portion of rind	Water		Reducing sugar		Invert sugar		Total sugar		Naringin	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Stem end	81.5	80.3–82.5	25.23	22.70–26.82	12.18	8.35–17.00	37.41	35.16–39.69	7.31	4.62–8.31
Blossom end	78.2	72.9–79.5	24.62	23.41–26.27	11.50	7.85–15.59	36.12	34.12–39.00	7.32	5.51–8.36

Portion of rind	Hydrolyzable polysaccharides		Soluble solids		Insoluble solids		Hydrogen-ion concentration		Flavedo-albedo ratio	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*pH* [2]	*pH*	*Pct.*	*Pct.*
Stem end	7.32	6.03–9.68	70.2	65.0–73.3	29.8	26.7–35.0	6.45	6.25–6.66	0.74	0.68–0.78
Blossom end	7.45	6.11–9.12	69.5	63.2–74.1	30.5	25.9–34.8	6.40	6.29–6.59	.75	.65–.81

[1] All values except those for the water, hydrogen-ion concentration, and flavedo-albedo ratio are calculated on the basis of dry weight.

[2] The writers are fully aware that the averaging of pH values, here and elsewhere in this paper, is mathematically unsound, but they believe that through the ranges shown the averages of pH values as numerical entities are probably as sound biologically as the averages of the actual hydrogen-ion concentrations or the equivalent pH values, and are considerably more convenient.

TABLE 2.—*A comparison of the chemical composition of Marsh grapefruit stem-end and blossom-end rinds at Corona and Fontana and at Oasis, Calif., during the season of 1933–34*—Continued

OASIS, 1933-34

Portion of rind	Water		Reducing sugar		Invert sugar		Total sugar		Naringin	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Stem end	77.4	74.7–81.2	20.73	14.95–27.42	9.04	7.09–12.60	29.77	22.29–34.51	6.76	4.68–9.84
Blossom end	76.3	73.0–80.1	20.42	13.50–26.65	10.33	7.77–15.39	30.75	21.27–36.61	6.17	4.69–8.57

Portion of rind	Hydrolyzable polysaccharides		Soluble solids		Insoluble solids		Hydrogen-ion concentration		Flavedo-albedo ratio	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*pH*[1]	*pH*	*Pct.*	*Pct.*
Stem end	11.92	9.44–15.50	60.9	51.4–65.4	39.1	34.6–48.6	6.24	5.78–6.79	0.81	0.75–0.88
Blossom end	11.34	8.30–14.26	61.2	53.7–68.3	38.8	31.7–46.3	6.22	5.70–6.61	.89	.82–1.04

[1] The writers are fully aware that the averaging of pH values, here and elsewhere in this paper, is mathematically unsound, but they believe that through the ranges shown the averages of pH values as numerical entities are probably as sound biologically as the averages of the actual hydrogen-ion concentrations or the equivalent pH values, and are considerably more covenient.

Naringin and the flavedo-albedo ratio were approximately the same in both rind portions.

The total average difference for all substances in the two ends was 3.4 percent. The corresponding average differences in the Valencia and Washington Navel orange rinds were about 13 and 11 percent, respectively.

AT OASIS

Table 2, which also presents the data for the stem and blossom ends of the rind of Marsh grapefruit from Oasis, shows that the difference between the two ends was even less than that in the rind of grapefruit from the Fontana-Corona district. The total average difference was only 1.3 percent.

COMPARISON OF FLAVEDO AND ALBEDO

Table 3 shows the chemical composition of the flavedo and albedo of grapefruit from Fontana and Corona. The differences in composition of these tissues were very small, the total average difference being only 2.2 percent. The corresponding difference between the flavedo and albedo for Valencia and Washington Navel oranges (*13*) had been found to be about 10 percent and 16 percent, respectively.

Table 3 also offers a comparison of the flavedo and albedo of grapefruit from Oasis. The two tissues show significant differences in regard to some substances. The flavedo was considerably higher than the albedo in invert sugar, total sugar, and soluble solids. The albedo was higher in naringin, hydrolyzable polysaccharides, and hydrogen-ion concentration. The total average difference was 8.3 percent, or nearly equal to the corresponding difference in Valencia oranges (*13*).

TABLE 3.—*A comparison of the chemical composition of grapefruit flavedo and albedo at Corona and Fontana, and at Oasis, Calif., during the season of 1933–34* [1]

CORONA AND FONTANA, 1933

Portion of rind	Water		Reducing sugar		Invert sugar		Total sugar		Naringin	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Flavedo	79.4	77.5–80.8	25.00	22.70–27.10	11.79	7.93–15.75	36.79	35.03–39.04	6.38	4.17–7.87
Albedo	80.3	79.6–80.9	24.85	22.74–26.23	11.89	8.27–16.84	36.74	34.26–38.39	8.26	5.96–10.35

Portion of rind	Hydrolyzable polysaccharides		Soluble solids		Insoluble solids		Hydrogen-ion concentration		Flavedo-albedo ratio	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*pH*	*pH*	*Pct.*	*Pct.*
Flavedo	6.26	5.42–7.48	71.4	68.3–74.8	28.6	25.2–31.7	6.72	6.52–6.94	0.74	0.71–0.77
Albedo	8.51	6.88–11.31	68.3	61.9–72.5	31.7	27.5–38.1	6.14	5.96–6.30	-----	-----

OASIS, 1933–34

Portion of rind	Water		Reducing sugar		Invert sugar		Total sugar		Naringin	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Flavedo	76.7	72.7–81.3	20.06	11.58–27.73	10.94	5.37–15.26	31.00	16.95–36.15	4.80	3.01–8.24
Albedo	77.0	74.6–80.0	21.09	16.87–26.33	8.43	5.01–12.73	29.52	25.52–32.60	8.15	6.85–10.17

Portion of rind	Hydrolyzable polysaccharides		Soluble solids		Insoluble solids		Hydrogen-ion concentration		Flavedo-albedo ratio	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*pH*	*pH*	*Pct.*	*Pct.*
Flavedo	10.27	7.46–14.09	64.3	53.8–70.1	35.7	29.9–46.2	6.54	5.88–7.07	0.81	0.75–1.04
Albedo	12.99	10.28–15.67	57.7	51.3–63.4	42.3	36.6–48.7	5.93	5.60–6.34	-----	-----

[1] All values except those for the water, hydrogen-ion concentration, and flavedo-albedo ratio are calculated on the basis of dry weight.

It is interesting to note that the grapefruit from Oasis showed less difference between the stem-end and blossom-end rinds and more difference between flavedo and albedo than did the grapefruit from Fontana and Corona. The same relation was noted in a similar comparison between Valencia and Washington Navel orange rinds (*13*).

COMPARISON OF FRUIT FROM DIFFERENT LOCALITIES

Table 4 presents a comparison of the composition of grapefruit rinds from the three localities from which fruits were obtained. The

Fontana and Corona values were averaged, as shown in the third line of table 4, in order to be in agreement with the other comparisons in this study.

TABLE 4.—*Comparison of the chemical changes in the rind of Marsh grapefruit in different localities during the season of 1933* [1]

Locality	Water	Reducing sugar	Invert sugar	Total sugar	Naringin	Hydrolyzable polysaccharides	Soluble solids	Insoluble solids	Hydrogen-ion concentration	Flavedo-albedo ratio
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*pH*	*Pct.*
Corona	80.00	24.02	12.62	36.64	7.64	7.40	70.00	30.00	6.30	0.77
Fontana	79.70	25.83	11.07	36.90	6.98	7.37	69.70	30.30	6.55	.71
Average	79.85	24.93	11.85	36.77	7.31	7.39	69.85	30.15	6.43	.74
Oasis	76.80	20.58	9.69	30.27	6.47	11.63	61.00	39.00	6.24	.81

[1] All values except those for water, hydrogen-ion concentration, and flavedo-albedo ratio are calculated on the basis of dry weight.

When the individual values of the three localities are examined separately, it becomes evident that the Fontana and Corona rinds agree more closely in composition than either agrees with the Oasis rinds. This fact tends to justify the present plan of handling the Fontana and Corona data together and contrasting them with the Oasis data. Of the two former, however, the Corona showed a slightly closer resemblance to the Oasis rinds.

The average of the Fontana and Corona rinds was higher than that of the Oasis rinds in water, reducing sugar, invert sugar, naringin, and soluble solids. This higher sugar content of the Fontana and Corona rinds was rather surprising. The Oasis rinds were higher in hydrolyzable polysaccharides and hydrogen-ion concentration, and had a larger flavedo-albedo ratio.

DISCUSSION

In the foregoing presentation of data on the composition of the Marsh grapefruit rind consideration has been given to seasonal changes, differences between stem and blossom ends, flavedo and albedo, and the possible influence of local or climatic environment. Measurable changes and differences were noted in most of these comparisons. In this and in another report on citrus rinds (*13*), the writers have placed some emphasis upon such differences because of the possible relation of some of them to subsequent pitting and spotting of citrus rind in storage or during transportation. For example, it has been pointed out that if the stem-end rind differed considerably in composition from that of the blossom end and was much more subject to spotting, then those differences in composition might be associated with the cause or causes of the trouble. Again, corresponding differences in the composition of the flavedo and albedo might be of importance in determining why pitting or spotting originated in one or the other of these tissues.

In the comparison of stem- and blossom-end rinds, it was noted not only that grapefruit showed less difference between these portions than did oranges, but also that while the difference was still noticeable in the Fontana-Corona rinds it was very small in the Oasis rind. But in the comparison of flavedo and albedo it was noted that the difference between these tissues was greater at Oasis than at Fontana

and Corona, and less in rinds of fruit from all these localities than in the orange rinds.

That there can be no hard and fast interpretation of such differences as may be recorded in total seasonal averages seems obvious. But there may be something of considerable value, theoretically, in observing variations in the differences between the various tissues in relation to changes in the season and in the immediate environment.

The matter of this seasonal difference between rind tissues has been discussed in the report on orange rinds (*13*). It is referred to again because the graphs representing these seasonal differences (figs. 5 and 6) show how extremely variable are the relations in regard to composition between any two tissues of the rinds under comparison.

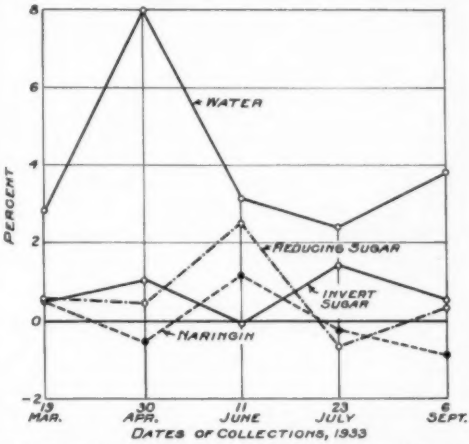


FIGURE 5.—Seasonal changes in the differences between the average composition of the stem end and blossom end of the rind of Marsh grapefruit from Corona and Fontana, Calif., with respect to water, reducing and invert sugar, and naringin. Positive values indicate the stem end higher than the blossom end and negative values the reverse.

The average value of the difference for the season as a whole is often small as compared with differences that may appear at various times during the season. Changes may occur in the rind at a given period that are a complete reversal of the trend as indicated by the seasonal averages.

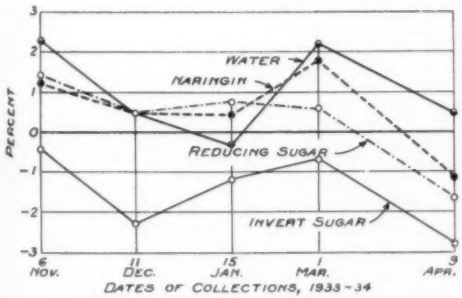


FIGURE 6.—Seasonal changes in the differences between the average composition of the stem end and blossom end of the rind of Marsh grapefruit from Oasis, Calif., with respect to water, reducing and invert sugar, and naringin. Positive values indicate the stem end higher than the blossom end and negative values the reverse.

The Fontana-Corona average seasonal difference in the two ends was recorded as 3.4 percent, yet the graphs of figure 5 show generally less than this figure. The much larger percentage of water in the stem end helped greatly to increase the seasonal average difference. At the second collection the difference in water content between the rind of the ends of the fruit was about 5 percent greater than the normal seasonal difference. Naringin was higher in the stem end at the first and third collections, but lower at the second, fourth, and fifth collections. Reducing sugar, usually higher in the stem end, was lower at the fourth collection.

At Oasis, where the average seasonal difference between stem end and blossom end was 1.3 percent, an amount scarcely considered significant, figure 6 shows that there were many variations in respect to individual differences. The tissues show a different relation to each other in respect to composition at every collection date. Naringin, which was higher in the stem end through four collections, was lower at the fifth collection. Water and sugars varied constantly in their interrelationships.

The foregoing data indicate that, when it is said that one portion of the rind of a citrus fruit contains more of a certain substance than another, it should be made plain whether one is speaking generally or of specific instances. Apparently almost any relationship can be found between rind tissues on occasion, and these occasional changes may have much to do with the subsequent behavior of citrus rinds. It seems clear that the interrelationships of substances in the various portions of the rind can be modified relatively rapidly and significantly by weather conditions.

STORAGE STUDIES

So far no reference has been made to findings that might relate time of picking, composition of rind, location, etc., to pitting and spotting of grapefruit in storage. The rinds used in the studies just reported were analyzed directly from the field, and therefore had no opportunity to develop spotting. However, for each lot of fruit used in the field studies a corresponding lot was collected and placed in storage. At the end of the storage periods these were inspected and later analyzed. The principal aims of these studies were (1) to determine some of the general chemical changes occurring in grapefruit rinds during storage, (2) to note the relation of such changes to the previously determined condition of the rinds at the time of picking, (3) to correlate the findings with the susceptibility of the rinds to pitting and spotting, and (4) to study the effects of various substances and treatments on pitting and spotting.

MATERIALS AND METHODS

The grapefruit used in the main storage experiments were from the same selected Marsh grapefruit trees that furnished the material for the studies of rinds in the field. At the time that most of the field collections of grapefruit for immediate study were made, additional lots were taken for storage under different conditions. The relation of the former to the latter and to the season is shown in figure 7.

The storage temperatures employed were 32° to 33°, 42°, and 51° to 52° F.; they fluctuated through a relatively narrow range, the greatest variation being in the 32° to 33° room. Throughout the paper the temperatures are referred to simply as 32°, 42°, and 52°, unless there is need to mention the exact temperature. In the three storage rooms an average relative humidity of 84, 87, and 89 percent, respectively, was maintained. The length of the storage periods was generally about 6 weeks. At the end of that time inspections were made for pitting and spotting, and the rinds were prepared for analysis.

The methods of preparing and analyzing the rinds were identical with those described in previous reports (*13*, *14*, *24*). As in the field

studies, the rind was separated into four parts, as follows: Stem-end flavedo; stem-end albedo; blossom-end flavedo; and blossom-end albedo. The analyses of the rinds included the determination of water, soluble and insoluble solids, reducing sugar, invert sugar, naringin, hydrolyzable polysaccharides, and hydrogen-ion concentration. The juice from the pulp was examined only for hydrogen-ion concentration and titratable acidity.

MAIN STORAGE EXPERIMENTS

Two collections for storage comprising three lots each were made during the season at Corona and Fontana, and five were made at

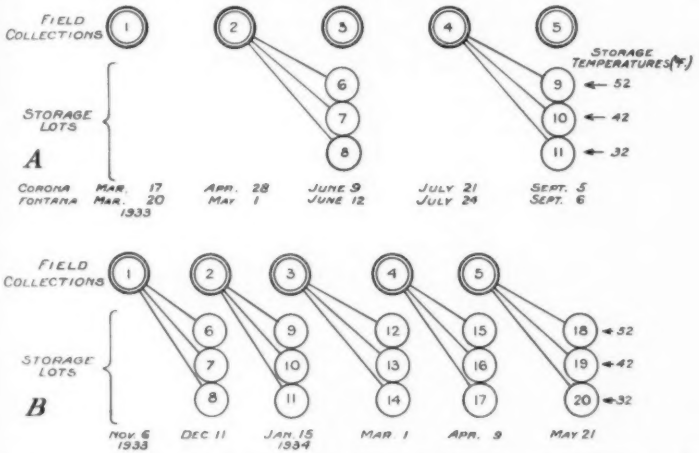


FIGURE 7.—Diagram showing relationship of storage lots to the various field collections: *A*, Corona and Fontana; *B*, Oasis.

Oasis. In fact, at the latter location there was a collection for storage corresponding to every field collection for immediate use.

The results of the investigations of the rinds of these grapefruit after storage will be presented separately for the two principal districts involved. The Corona and Fontana grapefruit rinds behaved very much alike qualitatively in almost every detail noted. On the other hand, the behavior of the rinds of the Oasis fruit differed in several respects from those of Corona and Fontana.

DESCRIPTION OF THE STORAGE LOTS AND INSPECTIONS

The first Corona and Fontana storage lots correspond to the second immediate field collections picked April 28 and May 1 in the two respective locations and placed directly in storage. The fruit was firm and well-colored when picked, but could not be considered further advanced than early maturity. At the time of removal from storage the fruit was still firm in all three storage rooms. The effects of temperature and other storage conditions on pitting and spotting of the rinds are presented in table 5.

TABLE 5.—*Effect of storage temperature and date of picking on pitting and spotting of Marsh grapefruit grown at Corona and Fontana and at Oasis, Calif., 1933–34*

CORONA AND FONTANA, 1933

Date of collection	Location	Lot no.	Storage period	Storage temperature	Relative humidity		Pitting and spotting		
					Weighted average	Range	Slight[1]	Severe[1]	Total
1933			*Days*	*° F.*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Apr. 28	Corona	6	42	51	88	84–92	5	0	5
		7	42	42	85	79–89	13	50	63
July 21		8	42	32	84	83–90	30	0	30
		9	46	52	87	82–94	16	0	16
		10	46	42	85	78–89	30	33	63
		11	46	33	79	75–85	10	7	17
May 1	Fontana	6	42	51	88	84–92	5	0	5
		7	42	42	85	79–89	24	76	100
July 24		8	42	32	84	83–90	3	27	30
		9	44	52	87	82–94	10	0	10
		10	44	42	85	78–89	31	60	91
		11	44	33	79	75–85	51	21	72

OASIS, 1933–34

Nov. 6	Coachella Valley	6	33	52	89	87–94	0	0	0
		7	33	42	87	83–91	0	100	100
		8	33	33	86	79–91	0	0	0
Dec. 11		9	37	52	94	92–95	0	0	0
		10	37	42	86	76–90	25	25	50
1934		11	37	32	81	76–89	0	0	0
Jan. 15		12	44	52	89	80–90	0	0	0
		13	44	42	88	79–91	22	8	30
Mar. 1		14	44	33	88	82–93	6	12	18
		15	39	51	89	84–93	0	0	0
		16	39	42	89	85–92	34	8	42
Apr. 9		17	39	33	88	83–92	35	18	53
		18	42	52	90	86–94	0	0	0
		19	42	42	93	91–95	64	28	92
		20	42	33	89	85–91	54	44	98

[1] Slight pitting and spotting includes only such cases as were not considered of commercial importance. Severe pitting and spotting includes all cases which might affect the commercial value of the fruit. A rather arbitrary standard involving both size and number of spots had to be adopted for such judgments.

The second Corona and Fontana storage collections (lots 9 to 11) correspond to the fourth field collections for immediate study. They were picked July 21 and July 24 at the two respective locations and placed in storage at once. The fruit when picked was somewhat less firm than the previous collection because of its considerably advanced maturity and the prevailing warmer weather. It was also less firm at the end of the storage period, but was not seriously shrunken. The condition as regards pitting and spotting is given in table 5.

The first Oasis storage collection was picked November 6 and placed in storage the same day. It corresponds to the first field collection for immediate study. The fruit was rather dark green, and the date of picking preceded the first commercial picking by about 3 weeks. The fruit was firm when removed from storage, and that which had been at 52° F. was beautifully colored and entirely free from storage blemishes. That from 42° was severely pitted and spotted, and in the occasional areas free from these blemishes there was considerable green color remaining. That from 32° was almost as green as when placed in storage, but entirely free from storage pits or spots. Plate 1, *A–C*, shows the appearance of these lots after storage. The results are given numerically in table 5.

The second Oasis storage collection was picked December 11 and placed in storage the same day. The color of the fruit was light green, and commercial picking had been in progress about 3 weeks. On removal from storage the fruit that had been held at 52° F. was a clear yellow and entirely free from storage blemishes; that held at 32° still retained some green color but was also free from blemishes; but that held at 42° was badly pitted and spotted, although less so than the corresponding first storage collection (table 5).

The third Oasis storage collection was picked January 15 and placed in storage the same day. The fruit was fully colored and firm, and represented prime harvest maturity. At the end of the storage period it was the least pitted and spotted of any used in the Oasis storage experiments (table 5).

The fourth Oasis storage collection was picked March 1 and stored the same day. The fruit was not so firm as that of previous collections, but still was in very good condition. In storage the spotting had become rather serious at 32° F. and the injury had increased somewhat at 42° (table 5).

The fifth Oasis storage collection was picked April 9 and stored the same day. The fruit was noticeably past prime harvest condition. The effects of higher air temperatures and advanced maturity were indicated by lack of firmness, large sizes, and "hollow cores." In storage this fruit pitted and spotted almost as badly as the November collection (table 5), although the number of "severe" cases was not so large. The response of the Oasis grapefruit rinds to the storage conditions furnished is shown in figure 8.

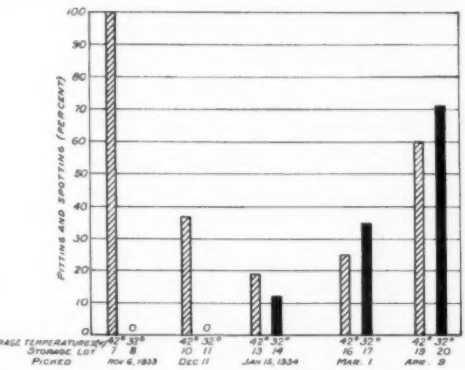


FIGURE 8.—Seasonal changes in susceptibility to pitting and spotting of Marsh grapefruit from Oasis after storage at 32° and 42° F. No pitting or spotting at 52°.

RELATION OF PITTING AND SPOTTING TO TIME OF PICKING AND TEMPERATURE OF STORAGE

The variation in the effects of the different storage temperatures with the season of picking was very interesting. In the fruit from Oasis at 52° F. there was no pitting or spotting of grapefruit picked at any stage of maturity; but at 42° every fruit of the November collection was severely affected with storage spots. The December collection had about one-half as much spotting as the first, but not all the fruits involved were seriously affected. The January collection showed the least susceptibility to spotting but in later collections spotting increased greatly.

The same variation in susceptibility to storage spotting with variations in temperature and stage of maturity was indicated by the behavior of the two storage collections from Corona and Fontana. The

first collection from both localities showed a very high susceptibility to spotting at 42° F., but low at 32°, but in the second collection spotting increased greatly at 32° in the Fontana fruit. There were only traces of pitting and spotting at 52° in any of the Corona and Fontana fruit. These two storage collections are believed to correspond as regards maturity to the second and fourth Oasis collections.

It should be emphasized that the types of spotting or pitting which occurred at 42° and 32° F. were noticeably different. The spotting or pitting at 32° usually involved very small islands of tissue. These were similar to the characteristic "pock" or the small, sharply sunken, but almost colorless blemishes commonly occurring on California grapefruit exported to England. Another type of spotting at 32° was small, shallow, and reddish in color. In one lot at 32° there developed a sort of soft scald. The spotting or pitting occurring at 42°, however, usually involved large irregular areas which were more or less discolored and often showed concentric rings marking their development. In addition, coalescence of spots frequently occurred.

When the grapefruit was brought from storage at 32° to 33° F. and held either at room temperature or at 52° for a few days, it usually developed severe spotting. These spots resembled the characteristic 42° rather than the 32° spots.

Taking the results of the storage tests as a whole, there is little doubt that a temperature of 40° to 42° F. produced more spotting of California Marsh grapefruit than the other temperatures used. This conclusion seems to be corroborated by the unpublished observations of a number of other workers. Although temperatures below 42° may not produce so much spotting in storage, they tend to render grapefruit more susceptible to spotting after removal to higher temperatures.

For more than a decade workers (*1*, *8*, *9*, *14*, *15*, *16*, *19*) in various parts of the country have attempted to determine the best storage conditions for grapefruit. Such investigations have not led to a general agreement, and at present it seems very improbable that there will ever be a satisfactory recommendation that grapefruit should be held at a certain definite temperature and relative humidity. The situation is too complex to have a simple solution. Some of the important factors to be taken into consideration are variety, the section of the country where the fruit is grown, the districts within those sections, the cultural treatment of the grove, stage of maturity of the fruit, weather conditions at time of picking, and the various packing-house treatments. The importance of most of these factors is now recognized and some effort is being made to integrate them sufficiently to allow special and practical recommendations to be made, at least for the different citrus-growing districts. The readiness with which grapefruit from the different districts become infected by mold and other organisms may sometimes require the recommendation of a storage temperature known to be far from ideal for the best control of storage spotting. The same procedure may be necessary in order to avoid the development of too deep a color in storage at the higher temperatures.

Considering only the physiology of California Marsh grapefruit rinds as regards spotting and pitting in storage, there remains little doubt that a temperature of 50° to 55° F., or somewhat higher, and a relative humidity of approximately 90 percent would be much better than anything below these values. These results are in agreement with those of Brooks and McColloch (*1*) for Florida grapefruit.

Chemical Analysis

CORONA AND FONTANA

In table 6 are summarized the chemical changes recorded for the Corona and Fontana grapefruit rinds in storage at different temperatures. For each storage temperature the data presented are the combination of both storage collections from the two localities and for all portions of the rind. Each figure in the table is the average of 32 determinations.

TABLE 6.—*Effect of storage temperature on the composition of the rind of Marsh grapefruit grown at Corona and Fontana, Calif.*

[Percentages except water are on dry-weight basis, each figure being the average of 32 determinations]

Item	No storage	52° F.	42° F.	32°–33° F.
Water.....Percent..	80.1	79.1	79.6	79.5
Reducing sugar.....do....	24.58	29.52	30.33	23.85
Invert sugar.....do....	12.91	2.69	3.60	11.40
Total sugar.....	37.49	32.21	33.93	35.25
Naringin.....do....	8.03	7.40	6.91	6.38
Hydrolyzable polysaccharides.....do....	7.28	7.56	7.41	7.44
Soluble solids.....do....	70.5	68.1	68.4	69.6
Insoluble solids.....do....	29.5	31.9	31.6	30.4
Flavedo-albedo ratio.....do....	.76	.76	.71	.72
Rind.....pH..	6.40	6.10	5.97	6.29
Pulp juice.....pH..	3.09	3.08	3.06	3.09
Acidity [1].....ml..	3.33	3.30	3.30	3.17

[1] As milliliters of N/1 acid per 10 ml of juice.

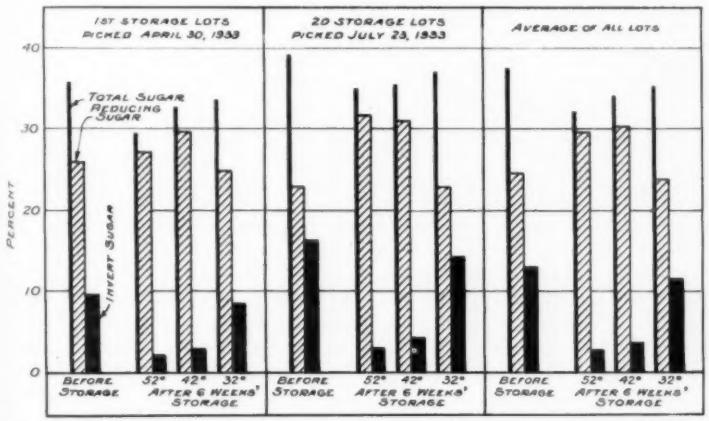


FIGURE 9.—Effect of storage temperature on the sugar content of the rind of Marsh grapefruit picked at different times; average of fruit from Corona and Fontana.

The water content of the rinds changed only slightly during storage, but tended to decrease. Total sugar invariably decreased, this decrease being least at 32° F. and greatest at 52°. This and other points referred to below are shown graphically in figures 9 and 10. Reducing sugar always increased at 52° and 42°, the increase being

about the same at both temperatures (fig. 9). At 32° there was little change in the amount of reducing sugar, although a slight decrease was noted.

Invert sugar decreased strikingly at all temperatures except at 32° F., at which the decrease was slight. Although the difference in temperature between the storage rooms was 10° the amount of inversion of sucrose [3] was nearly the same at 52° and 42° but very much less at 32°. Apparently there is some critical point lying between 32° and 42° where the action of invertase in the rind tissue is

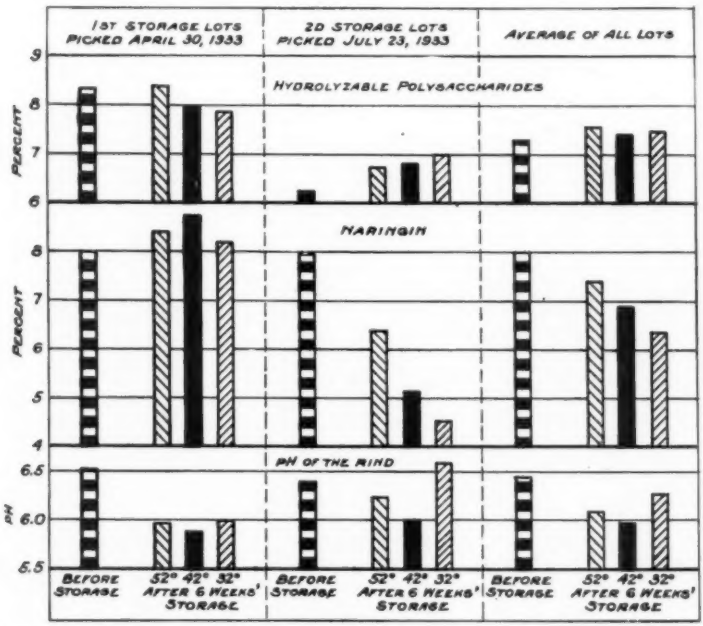
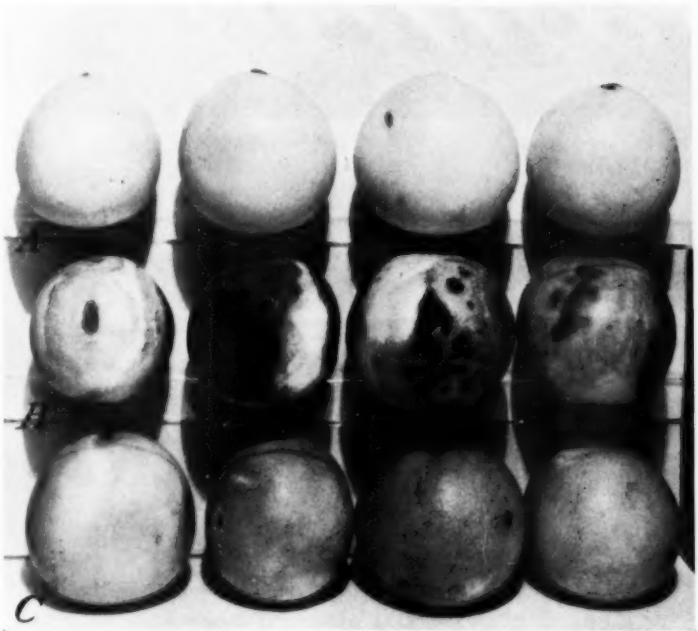


FIGURE 10.—Effect of storage temperature on hydrolyzable polysaccharides, naringin, and hydrogen-ion concentration of the rind of Marsh grapefruit picked at different times; average of fruit from Corona and Fontana.

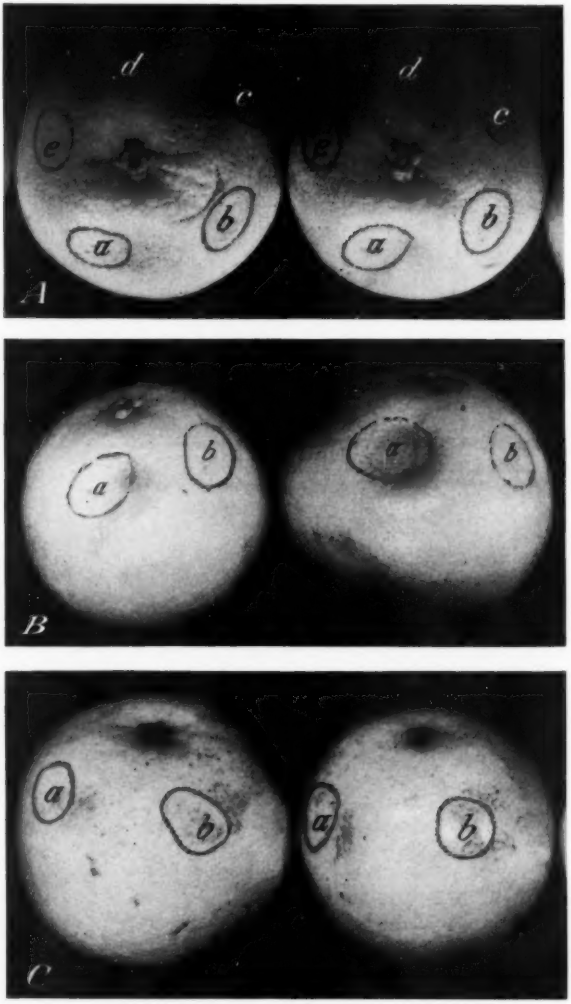
strongly inhibited. Invertase, acting upon sucrose solutions in vitro at the three storage temperatures, did not show a corresponding break between 32° and 42°. At the two higher temperatures inversion apparently proceeded faster than the products were used in respiration—a supposition that accounts for the increase of reducing sugars noted at these temperatures. The same behavior of the different sugars in storage as outlined above was noted in every portion of the rind for both localities. The actual amount of inversion at the higher storage temperatures depended on the original sucrose content. The higher the latter the more the inversion, but the percentage of sucrose that remained was practically always the same regardless of the original content.

[3] The term "sucrose" has generally been avoided; however, the writers are certain that "invert sugar" of this report is derived almost wholly from sucrose.

Changes in Composition of Rind of Marsh Grapefruit PLATE 1



Marsh grapefruit collected at Oasis (Coachella Valley) November 6, 1933, and stored for 33 days: *A*, At 52° F., the spots showing being tree blemishes, as no storage spot occurred; *B*, at 42°, all fruit spotted badly; *C*, at 32°, at which no storage spot occurred, the spots showing being tree blemishes and the shading due to the unequal distribution of the green color remaining on the fruit.



A, Marsh grapefruit from Claremont 9 days after being injected with 2 ml each of the following solutions and held at 42° F.: *a*, 0.1-percent naringenin; *b*, 0.028-percent naringenin; *c*, weak ferric chloride; *d* .5-percent alcohol; *e*, 0.1-percent naringin. *B*, The same fruit as in *A*, after being held 24 hours at room temperature; *a*, 0.1-percent naringenin and, *b*, 0.028-percent naringenin. *C*, Marsh grapefruit from Claremont 2 weeks after being injected; *a*, 0.6 mg of naringenin and *b*, 0.3 mg of naringenin, and held at 42° F.

Naringin in the Corona and Fontana rinds usually decreased at all temperatures (fig. 10), the decrease being greatest at 32° F. and least at 52°. Naringin, however, does not always decrease in storage. In a few instances it increased in the Corona and Fontana rinds, and as will later be shown, the synthesis of naringin in the Oasis rinds in storage was the usual occurrence. Zoller (*24*) reported a rapid hydrolysis of naringin in grapefruit during storage, but the conditions of storage were not given. It has since been stated repeatedly by Hawkins (*14, 15*) and others (*7, 18*) that naringin decreases in storage, but without indication that actual determinations of naringin had been made. The studies of the writers on the behavior of naringin and hesperidin, and those of the senior writer on phloridzin, have shown that in living tissue separated from the tree, the phenolic glucosides will be hydrolyzed or synthesized, depending upon certain conditions which are poorly understood. The difficulty of understanding seems to lie in the fact that a seemingly very slight change in the environment may determine the direction of the reversible reaction. The condition of the tissue when collected is apparently an important determinant of the subsequent behavior of the glucoside. In any case no one can say to date that naringin has decreased in storage without determining it in each instance.

Determinations of the hydrogen-ion concentration showed that active acidity increased, except in a few instances, at 32° F. At 42° the increase was greatest in such a large proportion of cases that the tendency may be considered as unmistakable. It was more or less expected that the increase in acidity would be least at 32° and greatest at 52°, but that it should be greatest at 42° was surprising.

During these studies a number of observations were made which indicate that the reactions going on in grapefruit rinds at 42° F. were very different from those at the other two temperatures. Of course, it is possible, and is sometimes indicated, that many of the same basic reactions may go on at 32° as at 42°, but if so they are probably unable to manifest themselves at the lower temperature. These facts appear to have special significance in view of the further fact that storage pitting or spotting was much more severe at 42°. However, it is not intended to imply that any of the chemical or physical reactions observed as happening at 42° are directly responsible for the storage pitting or spotting, although they may be associated in the same complex of reactions that cause the storage maladies.

The increased acidity at 42° F. is particularly interesting because high acidity was positively correlated with naringin content in all field and storage lots from every locality.[4] The higher the acidity the higher was the coefficient of correlation. This may indicate only that both naringin and active acidity are at times fairly good indexes of the relative rate of metabolism.

Hydrolyzable polysaccharides changed very slightly in any of the lots (fig. 10), but apparently they decreased during the storage period. Soluble solids gave about the same results.

A comparison of the chemical composition of the flavedo and albedo and of the stem and blossom ends of grapefruit rinds in the field was given earlier in this paper. The data from the present storage work

[4] The following coefficients of correlation of naringin and active acidity may be of interest: Field and storage samples of Corona and Fontana gave $r=0.61\pm0.05$; storage only, Corona and Fontana, $r=0.70\pm0.05$; and field and storage lots at Oasis, $r=0.88\pm0.03$.

showed the relations between the two tissues and the two end rinds to be so nearly like those described for the field samples used for immediate study that a separate presentation of a corresponding set of comparisons seems unnecessary.

The rinds of fruit from Corona and Fontana had much the same chemical composition and behaved similarly in storage. In general, the Corona rinds had slightly more invert sugar and less reducing sugar than the Fontana rinds. Active acid, naringin, and other substances examined were practically the same in the rinds from each locality. In storage the responses of these substances to the different temperatures were essentially the same. The rinds in the first storage experiment from both localities showed a slight tendency to synthesize naringin in storage, that is to say, naringin increased somewhat at 52° and 42° F. in the Corona rinds, but such differences are of doubtful importance, except as showing that small fluctuations

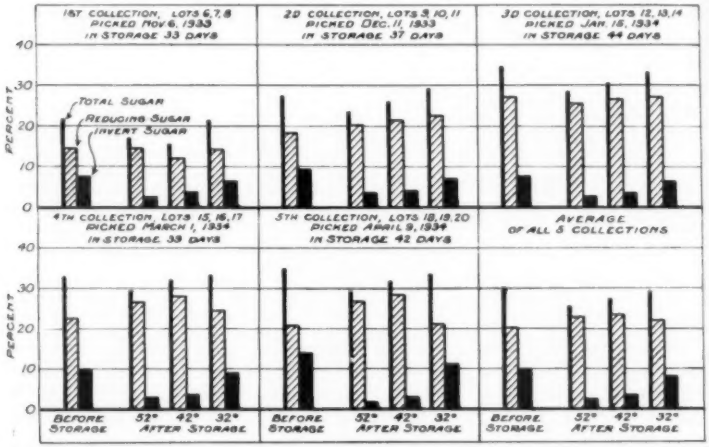


FIGURE 11.—Effect of storage temperature on the sugar content of the rind of Marsh grapefruit from Oasis.

were taking place in the equilibrium between naringin synthesis and hydrolysis. The possible consequences of a failure of the tissue to tie up naringenin, the derivative of naringin, is discussed later.

The relative susceptibility of the rinds of the two localities to pitting and spotting may be noted by referring again to table 5. Although the Fontana rinds show up a little worse than the Corona, the differences are such that macrochemical analyses cannot be expected to disclose what is really significant.

OASIS

The summarized data showing the changes that took place in the rinds of the Marsh grapefruit in storage, from Oasis, are presented in table 7. For each storage temperature the values given in the tables are the averages of all collections and all portions of the rind. Each figure is the average of 40 determinations.

Moisture conditions in the storage rooms were such that there was very little change in the water content of the rinds. At 52° F. there

was no appreciable change, and at 32° and 42° there was an average decrease of less than 1 percent during the storage period.

Total sugar decreased in storage at all temperatures (fig. 11).

TABLE 7.—*Effect of storage temperatures on the composition of Marsh grapefruit grown at Oasis, Calif.*

[Percentages except water are on dry-weight basis, each figure being the average of 40 determinations]

Item		No storage	52° F.	42° F.	32° F.
Water	Percent	76.8	77.0	76.1	76.0
Reducing sugar	do	20.67	22.89	23.45	21.86
Invert sugar	do	9.69	2.69	3.65	8.06
Total	do	30.36	25.58	27.10	29.92
Naringin	do	6.47	7.73	7.95	8.17
Hydrolyzable polysaccharides	do	11.62	11.63	11.33	11.13
Soluble solids	do	61.0	58.9	59.8	61.7
Insoluble solids	do	39.0	41.1	40.2	38.2
Rind	pH	6.23	5.90	5.91	6.04
Pulp juice	pH	3.28	3.35	3.28	3.33
Acidity [1]	ml	2.48	2.56	2.50	2.43

[1] As ml of N/1 acid per 10 ml of pulp juice.

Reducing sugar increased slightly at all storage temperatures (fig. 11 and table 7). The increase at 32° F. was largely at the expense of the polysaccharides, for the amount of inversion of sucrose at that temperature was probably not enough to satisfy the requirements for respiration. The increase of reducing sugar at the two other temperatures was due mostly to the rapid inversion of sucrose, for there was only a slight hydrolysis of polysaccharides at 42°. Although there was extensive inversion at 52°, the respiration at the higher temperatures prevented the increase in reducing sugar from exceeding the corresponding increase at 32°.

Invert sugar decreased at all storage temperatures (fig. 11). Inversion in the Oasis rinds was the same as in the Corona and Fontana rinds—that is, slight at 32° F. and rapid at 42° and 52°; also the rates were very similar at the last two temperatures.

Naringin increased at all temperatures (fig. 12). This synthesis of naringin was most pronounced at 32° F. and least at 52°.

It has been mentioned that naringin usually decreased in storage in the Corona and Fontana grapefruit rinds, and that the decrease was greatest at 32° F. The reversal of this entire situation at Oasis will be mentioned again, but it may be added here that the average change in naringin content of the different Oasis storage collections at all temperatures was in general in increasing order and that susceptibility to storage spotting was in the same but decreasing order with the exception of the fifth collection, which spotted nearly as badly as the first. The overmature fruit of the fifth collection behaved exceptionally in other ways, but the significance of the conditions which favor naringin synthesis in the rinds cannot be stated. The actual naringin content at the time of picking paralleled the susceptibility graph except for the fifth collection, when susceptibility increased and naringin decreased.

Soluble solids increased slightly at 32° F., but decreased at the other temperatures (table 7).

The active acidity of the rinds always increased in storage, most at 52° F. and least at 32° (fig. 12). The active acidity of the pulp juice in storage showed little change, but so far as could be noted it was downward.

The flavedo-albedo ratio increased at the two highest temperatures, but decreased at 32° F. These changes in value of the ratio seemed to be due mainly to the albedo, which often showed a greater versatility in response to different conditions than the flavedo.

OASIS COMPARED WITH CORONA-FONTANA

The data presented above deal entirely with the averages of all storage collections. Limitations of space prevent the presentation of the mass of data that would be required to show the size of deviations from the average condition that occurred in the rinds of individual

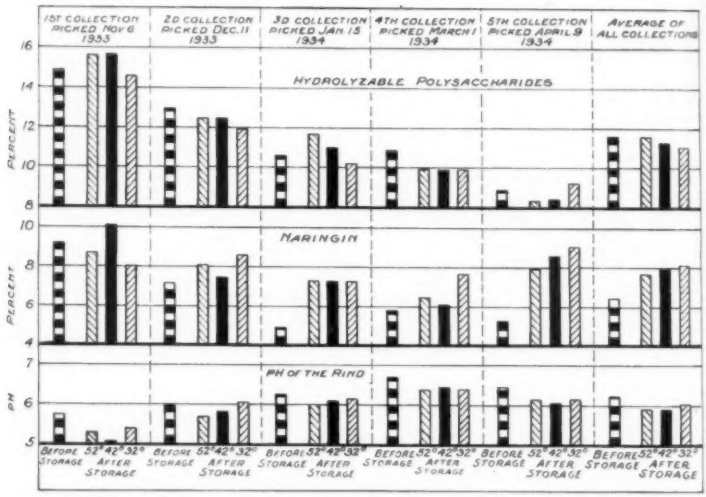


FIGURE 12.—Effect of storage temperature on hydrolyzable polysaccharides, naringin, and hydrogen-ion concentration of the rind of Marsh grapefruit from Oasis picked at different times.

collections or even in separate portions of the rinds in each collection. Figures 9 to 12, however, give a general idea of the variations between individual collections with respect to the substances represented. The first storage collection from each of the three localities showed a tendency to respond somewhat differently from the later collections. This is thought to have been due to the immaturity of the fruit at the time these collections were made, especially the first one at Oasis; otherwise a description of the behavior of the rinds of one collection would serve for the average of all. This statement is not so true for the separate portions of the rinds of individual collections in which there were a number of exceptions, but even for these it is true in the main. For example, naringin, which usually cannot be expected to behave in a very regular manner, not only showed a general tendency to be synthesized in the Oasis rinds in storage, but also responded in the same way in most of the portions of the rinds of all collections.

There were five exceptions in the stem-end rinds, three in the flavedo, and one in the albedo for all collections at all temperatures.

Total sugar in the Oasis rinds at the beginning of storage was about 7 percent less on a dry-weight basis (actually, not relatively) than in the Corona-Fontana rinds. This lower sugar content at Oasis was surprising, but the fact was tentatively attributed to the effect of conditions accompanying the winter harvest season as compared with the summer harvest season at Corona-Fontana. As compared with the fruit from the latter location, there was an average of more than 10 percent less water in the Oasis rinds at the beginning of storage; active acidity was also greater, but the naringin content was 1.50 percent lower. (See tables 6 and 7.) These and other differences in the original composition of the rinds may have been responsible for the differences subsequently noted in their behavior in storage.

Possibly the outstanding difference between the fruit from the two localities was the decrease of naringin in storage in the Corona-Fontana rinds and its corresponding increase in the Oasis rinds. The lower original sugar and naringin content, together with a higher active acidity in the Oasis rinds, might have been important factors in the observed synthesis of naringin in storage. The positive correlation between naringin content and active acidity was high, especially after storage, and this correlation was still higher in the above-average ranges of acidity in all collections and under all conditions of storage.

The hydrolyzable polysaccharides increased slightly in the Corona-Fontana rinds, but decreased at 42° F., and especially at 32°, in the Oasis rinds.

There were several other differences noted in the chemical responses of the grapefruit rinds from the two localities. The variations mentioned at least show that such tissues are capable of making opposite responses to a given experimental storage treatment and that the direction of the response is apparently determined by the internal condition of the tissue at the beginning of storage. These and other chemical responses recorded emphasize the possible variability of rind tissue and help toward a realization of the complexity of the problems encountered in the storage of citrus fruit.

MISCELLANEOUS STORAGE EXPERIMENTS

During the course of the main storage experiments an opportunity was found from time to time to carry out certain minor experiments suggested by apparent trends of the incoming data. However, because of the time elapsing between collections and the opportunity for examination of data from them, the larger number of these trends were observed too late to follow the leads with the same season's fruit. The minor experiments undertaken are reported below.

Transfer of Grapefruit From One Storage Temperature to Another

At the end of regular storage periods there were often extra lots of grapefruit which from time to time were transferred to a different temperature and held for further observation. The behavior of the rinds under such circumstances, as regards spotting, was sometimes rather striking. The previous storage conditions seemed to affect greatly the response to a second temperature. Of course this is a

situation quite generally known, but the details of such responses under given sets of conditions are of considerable interest. The only special undertaking by the writers to investigate this phase of the storage problem was one small series of storage experiments.

The grapefruit was collected at North Claremont, Calif., February 1, 1934, from a tree that belonged to a group previously selected to furnish fruit for other phases of the work. Nine lots of fruit were picked and placed in storage the same day at temperatures of 32°, 42°, and 52° F. Twenty-two days later all lots were inspected, then two lots from each storage room were transferred, one to each of the other two rooms. After 25 days more a final inspection was made. The results of both inspections are presented in table 8.

TABLE 8.—*Effects of transfer from one temperature to another on spotting of Marsh grapefruit in storage, using fruit grown at Claremont, Calif.*

Lot no.	Storage temperature	Spotting after 22 days			Lot no.	Temperature		Spotting after 25 days more		
		Severe	Slight	Total		From—	To—	Severe	Slight	Total
	°F.	Percent	Percent	Percent		°F.	°F.	Percent	Percent	Percent
1	52	0	0	0	1	52	52	0	0	0
2	52	0	0	0	5	42	52	15	19	34
3	52	0	0	0	8	32	52	0	15	15
4	42	0	28	28	4	42	42	18	19	37
5	42	0	31	31	2	52	42	0	5	5
6	42	0	15	15	9	32	42	10	29	39
7	32	0	0	0	7	32	32	14	14	28
8	32	0	0	0	3	52	32	0	5	5
9	32	0	10	10	6	42	32	29	24	53

It will be seen that there was very little spotting during the first storage period and that nearly all of it occurred at 42° F. No spotting was produced on the fruit held through both storage periods at 52°, and very little on the fruit held at 52° for the first storage period and then transferred to 42° and 32° for the second period. The fruit held at either 42° or 32° for the first period continued to spot after it was transferred to 52°. Lot 6, which was changed from 42° to 32°, fared somewhat the worst.

Gas Storage of Grapefruit

In reporting the acidity changes observed in grapefruit rinds during storage, attention was called to the fact that in an overwhelming proportion of the determinations the active acidity was greatest in rinds stored at 42° F., as compared with those at 52° and 32°. Pitting and spotting of the rinds was also most prevalent at 42°. Without assuming any causal relationship between these two factors, it was thought worth while to undertake a few experiments to determine the possible effects of some volatile acids and bases and other substances on the pitting and spotting of grapefruit in storage, on the chance that the production of these blemishes might be accelerated or retarded, depending on the reaction of the atmosphere surrounding the fruit. The results of these experiments are reported below.

The first experiment was started June 26, 1933. Grapefruit was picked from one tree of a selected group at Fontana. Six lots of fruit were enclosed in large glass desiccators and held in storage at

52°, 42°, and 32° F., there being two lots at each temperature. One lot was exposed to the fumes from an acetic acid solution (100 ml of glacial acetic in 2.5 l of water); the other was kept over water as a check.

On July 6 the fruit at 42° F. in the acidified chamber showed well-defined beginnings of pitting. Two days later pitting and spotting had increased at 42°, but there was none in any of the other lots. The final inspection was made July 28, with the following results: At 52° there was no pitting or spotting either in the acidified or the check lot; at 42° four of the check fruits were slightly pitted, but the acidified fruit was all badly pitted around the stem end; at 32° the checks showed no pitting or spotting, but the acidified fruit showed a few small pits and traces of brown stain or scald.

The acidified atmosphere seemed clearly to have aggravated the pitting of the rinds, especially at 42° F. The pitting or spotting which followed acidification of the surrounding atmosphere appeared similar to the usual storage pitting and spotting.

The hydrogen-ion concentration of the rinds of the six lots was determined after the gross inspection. Of the three check lots, that from 42° F. showed the highest active acidity. The acidified lot at 42° gave approximately the same value, but the acidified lots at 52° and 32° were higher in acidity than their corresponding check lots, but not so high as either of the 42° lots.

A second gas-storage experiment was started July 28, 1933. The fruit was from the tree used in the first experiment, and in all other respects this experiment was a duplicate of the first.

By August 3 all acidified fruit held at 42° F. had begun to pit. No other lots were yet affected. On August 5 the acidified lot at 32° showed slight pitting, and the pitting had increased at 42°. Final inspection was made September 19. At 52° the check lot was firm and sound, but all the fruit of the acidified lot was badly pitted and spotted; at 42° nearly all the check lot was slightly pitted, but the acidified lot was entirely and very severely pitted and spotted; at 32° one check fruit was slightly pitted, but the acidified fruit was all severely pitted, although not so severely as at 42°. All the check fruit from 32° pitted and spotted severely within 24 hours after removal from storage. The general results of this second experiment were practically the same as those of the first.

A third gas-storage experiment was started August 10, 1933. The chambers used were earthenware jars of 3-gallon capacity. Panes of glass sealed tightly to the tops of the jars by means of modeling clay served as covers. The fruit was from the same source and the storage temperatures were the same as in the preceding gas-storage experiments. There were 10 lots which received the following treatments: 3 check lots over water only; 3 lots over acetic acid solutions (same strength as before); 3 lots over dilute ammonia solution (5 ml of 28-percent NH_3 in 2.5 l of water); and 1 lot over a 6-percent ethyl alcohol solution. This last lot was stored at 42° F., and the others were distributed among the three storage temperatures. By August 21 the ammonified lots showed discolorations as a result of the too high concentration of ammonia, and the fruit was removed from these chambers and replaced with fresh fruit. The ammonia solution was then diluted to one-tenth its previous concentration. By

August 23 the acidified fruit at 42° and 52° had begun to pit. No other lots up to this time were affected.

The final inspection was made September 19, with the following results. At 52° F.: Check lot, one fruit slightly pitted; ammonified lot, all sound; acidified lot, all severely pitted and spotted. At 42°: Check lot, one fruit severely spotted; ammonified lot, four fruits slightly pitted; acidified lot, all fruits severely pitted and spotted; alcohol lot, four fruits slightly pitted. At 32°: Check lot, all sound; ammonified lot, all sound; acidified lot, all slightly to severely pitted or spotted.

The hydrogen-ion concentration of the rinds was determined after inspection. All lots were most acid at 42° F. The order of increasing acidity at every temperature was (1) ammonified, (2) checks, and (3) acidified. At 42° the lot over alcohol was the least acid of all at that temperature.

A fourth gas-storage experiment was started September 21, 1933. The grapefruit came from one of a group of selected trees near Corona. The arrangement of the different lots was the same as in the third experiment, except that there were nine chambers and 25-percent ethyl alcohol replaced the ammonia solution in the three corresponding lots.

On September 26 the fruit in the acidified lots at all temperatures had pitted, slightly at 32° F. but severely at the other temperatures. The acidified fruit was then removed from the three chambers, the acid solution replaced with ammonia solution (0.5 ml of 28-percent NH_3 solution in 2.5 l. of water), and fresh grapefruit was added. Final inspection was made November 16. It should be noted that the ammonified lots were in storage 5 days less than the others. The results of the inspection placed the lots in the following order as regards increasing amounts of pitting and spotting and other storage blemishes: 52°, check; 52°, ammonified; 42°, alcohol; and 42°, ammonified. The check and the lot over alcohol at 52° were in perfectly sound condition and the 42° ammonified lot was rather severely pitted and spotted.

The results of the foregoing experiments on grapefruit in atmospheres to which considerable quantities of certain substances had been added indicate that acetic acid accelerated and enhanced pitting or spotting, and that alcohol and very dilute ammonia retarded it. Alcohol seemed also to have retarded the development of yellow color in storage. The experiments were not extensive enough to allow a determination of optimum concentration of ammonia or alcohol, and further work on the problem might be justified.

Nelson (*19*) reported the results of experiments in which he enclosed oranges and grapefruit in jars with various organic chemicals, including a number of aldehydes, esters, and alcohols. In other experiments by various means he excluded oxygen from the fruit. Many of these treatments produced injuries to the rinds that resembled closely those which appear commonly in storage. The results of such tests and of those reported in this paper have no necessary connection with storage pitting and spotting. They do, however, demonstrate that if a cell, or an island of cells, of citrus rind is injured by almost any means, the manifestations of that injury are likely to resemble the blemishes which occur "naturally" in storage. The writers, in an

experiment with Valencia oranges, used 15 organic chemicals of the anesthetic type. The apparently characteristic types of storage injuries frequently occurred in the various lots, and in several instances (e. g., with ethyl chloride, ammonium thiocyanate, and ether) single lots exhibited almost the entire gamut of the common types of storage injuries.

Permeability

It seemed desirable to secure some idea of the relative permeability of grapefruit-rind tissue as affected by different temperatures and treatments. Some simple experiments were accordingly conducted. These were not designed to be critical permeability experiments, and perhaps the word "permeability" is inappropriate; the results obtained, however, seemed to indicate some definite effects of temperature on the integrity of rind tissue.

The first permeability study was made September 19, 1933, the fruit used being representative of each lot from the third gas-storage experiment after the final inspection. The stem-end rinds were taken for pH determinations and the blossom-end rinds for the permeability test. The albedo and flavedo of each storage lot were separated from each other, 75-g samples of each were weighed out, and then recombined. The resulting 150-g lots of fresh tissue were rinsed with distilled water and transferred to 1-liter Erlenmeyer flasks, and 750 ml of distilled water was added. The flasks were then placed upon a turntable which in revolving gently jolted and stirred the samples. The average temperature during the period of the experiment was about 70° F. After 24 hours the water was poured off through cheesecloth into 1-liter volumetric flasks and made up to volume. Then 900 ml from each flask were transferred to beakers and placed on a water bath to reduce the volume of the sample. Later these samples were transferred to evaporating dishes to complete evaporation and for final weighing. The flavedo and albedo tissues remaining in the flasks were discarded. After the weights of the solids which had exosmosed from each lot of tissue had been obtained, the solids were redissolved in water and made up to 1 liter for the determination of reducing sugar.

Table 9.—*Total solids and reducing sugars exosmosed from 150 g of living Marsh grapefruit rind after storage at different temperatures and atmospheres*

Storage temperature (° F.)	Treatment	Total solids	Reducing sugar	Storage temperature (° F.)	Treatment	Total solids	Reducing sugar
		Grams	*Grams*			*Grams*	*Grams*
52	Normal	11.33	1.96	42	Acidic	11.37	1.86
	Basic	10.95	1.65		Alcoholic	11.85	1.71
	Acidic	10.62	1.68	32	Normal	12.74	1.98
42	Normal	12.27	2.31		Basic	11.95	1.47
	Basic	11.53	1.89		Acidic	11.82	1.68

The results of the two operations described above are presented in table 9. The rinds from the 42° F. storage appeared to be the most permeable to reducing sugar, but the amount of total solids exosmosed was greatest from the 32° and least from the 52° storage tissue. The check lots seemed to have been most permeable to both reducing sugar and total soluble solids at all temperatures. The relative

positions of the various lots at each temperature do not seem definite enough for comment. This permeability test dealt with more factors than is usual in such experiments, particularly since the lots used had been held at various temperatures, so that there were differences among them in chemical composition aside from any permeability differences that may have been caused by differences in temperature.

A second permeability experiment was started October 27, 1933, with grapefruit from Corona and Fontana, which was picked on September 12 and 14, respectively. In the intervening time this fruit had been stored at 52°, 42°, and 32° F. The stem-end rinds only were used. The flavedo and albedo were separated, and 150 g of each constituted the samples, of which there were 12 (2 localities, 3 temperatures, and 2 portions of the rind). The samples were rinsed with distilled water. They were run on a turntable continuously for 68 hours at an average temperature of about 70°. After the jolting and stirring the solutions were filtered off. The amount of solution recovered from the tissues varied with the sample. The amount of water required to make the filtrates up to their original volume was always greater for the albedo than for the flavedo tissue, greatest for the 52° tissue and least for the 32°, and greater for the Corona than for the Fontana rinds.

The filtrates were evaporated to dryness and the amount of total soluble solids determined. The results are presented in table 10. It will be seen that the apparent permeability of the flavedo was greater than that of the albedo. It may have been, however, that the greater movement of water into the albedo tissue during the experiment tended to decrease the rate of diffusion of the solutes outward. The permeability, as regards total soluble solids, was again greatest at 32° F. and least at 52° for all situations except possibly one in the Fontana albedo.

TABLE 10.—*Total solids exosmosed by the flavedo and the albedo of 150 g of living Marsh grapefruit rinds after storage at different temperatures*

Storage temperature (° F.)	Corona		Fontana	
	Flavedo	Albedo	Flavedo	Albedo
	Grams	*Grams*	*Grams*	*Grams*
52	6.50	5.30	7.47	5.59
42	6.98	5.68	8.28	5.52
32	7.26	6.61	9.78	6.99

The third permeability experiment was started January 18, 1934, with grapefruit from Oasis, which was picked December 11, 1933, and placed in storage at 52°, 42°, and 32° F., where it remained until January 17. The rinds of the three storage lots were separated into stem-end flavedo, blossom-end flavedo, stem-end albedo, and blossom-end albedo. This division gave a total of 12 different samples of 150 g each. The samples were rinsed in water, transferred to 1-liter Erlenmeyer flasks, and 650 ml of distilled water was added. The flasks were then taken to a storage room in which the average temperature was about 34°. Into each flask was placed a glass tube which reached to the bottom, while the other end was connected by rubber tubing to a manifold having 12 outlets. The manifold was then

connected through a pressure-reducing valve to one of the compressed-air lines of the ice plant. Air was released from each outlet at the rate of about 5 l per hour. The bubbles of air rising through the tissue and solution in the flasks kept the samples both aerated and stirred. The decrease in volume of solution in the flasks, due to evaporation and absorption by the tissue, was made up to mark from time to time by additions of distilled water.

The experiment ran continuously until January 28, when the flasks were brought into the laboratory. The solutions were filtered off into 1-liter volumetric flasks and made up to volume. A 100-ml portion was taken from each flask for determination of reducing sugar and the remainder was used for total solids.

When the rind portions were removed from the flasks after the solutions had been poured off, they were moist-dried between filter paper and reweighed. The gain in weight of the tissue over the original 150 g plus the weight of the total solids exosmosed gave the amount of water absorbed by each lot during the experiment.

The results of the third permeability experiment are presented in table 11. The data show that the loss of reducing sugar and total soluble solids increased as the storage temperature was lowered. The amount of water absorbed by the flavedo tissue was in the same direction, but even more strongly marked. The absorption of water by the albedo did not vary much with the previous temperature treatment, although the total amount was nearly 10 times that absorbed by the flavedo. There seemed to be a somewhat greater permeability shown by the blossom-end flavedo and the stem-end albedo. Klotz and Haas (*16*) found a greater general permeability at the stem end of grapefruit. It has been shown that at the close of the storage period of the fruit used in these permeability tests the reducing sugar was considerably more abundant in the rinds held at 52° and 42° F. than in those held at 32°. Yet these last rinds subsequently lost more reducing sugar during the permeability tests than the 42° and 52° lots, thus indicating even more strongly that the greater permeability indicated at 32° was real.

TABLE 11.—*Total solids and reducing sugar exosmosed from, and amount of water absorbed by, stem- and blossom-end flavedo and albedo of 150 g of living Marsh grapefruit rinds after storage at different temperatures*

End	Storage temperature	Reducing sugar from—		Total solids from—		Water absorbed by—	
		Flavedo	Albedo	Flavedo	Albedo	Flavedo	Albedo
	°F.	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Stem	52	1.29	1.13	10.70	9.20	28.9	171
Blossom		1.37	----------	11.27	----------	28.5	178
Average		1.33	1.13	10.99	9.20	28.7	175
Stem	42	----------	1.45	----------	10.72	30.0	167
Blossom		1.70	1.33	12.27	9.78	31.6	195
Average		1.70	1.39	12.27	10.25	30.8	181
Stem	32	1.85	1.67	14.37	12.10	35.7	170
Blossom		1.95	1.54	14.55	9.91	33.1	184
Average		1.90	1.61	14.46	11.01	34.4	177

From these experiments as a whole, the conclusion is drawn that the permeability of the cells of grapefruit rinds to their own soluble constituents increased as the storage temperature was lowered. These findings are in agreement with those reported by Pantanelli (*20*) for endocarp tissue of the mandarin orange, although he worked at considerably lower temperatures. The increased permeability with the lowering of storage temperature might easily have an important bearing on the breaking down of fruit tissue in cold storage. The resulting increased ease of migration of certain harmful byproducts of metabolism through the tissue could allow the initiation of abnormal and injurious reactions.

Earlier in this paper the Corona-Fontana and the Oasis rinds were compared in respect to the behavior of naringin in storage. In the former rinds naringin was hydrolyzed, and in the latter synthesized, in storage. Both the hydrolysis and the synthesis were greatest at 32° F. The reasons for this latter situation are not apparent. It is possible, however, that the increased permeability found at the lower temperatures might have been partly responsible for it by permitting a more intimate association between the particular glucosidase and naringin. The increased permeability might thus offset the normal lowering of the activity of the enzyme, and so cause at 32° a more rapid hydrolysis or synthesis of naringin as the balance favored one or the other reaction.

Conductivity

When it was learned that the permeability of grapefruit rinds increased as the storage temperature was lowered it seemed desirable to determine what changes might take place in the conductivity of rinds under similar circumstances. Accordingly, a number of tests were carried out with grapefruit rinds from storage samples and from samples picked for immediate study. The instrument used was a Leeds and Northrup student potentiometer equipped for conductivity measurements, consisting of a microphon hummer, telephone receivers, and a conductivity cell of the dip type.

The first attempts were directed toward the measurement of the conductivity of the rinds in situ, but the results were so extremely variable that the method was abandoned. Subsequently the measurements were made on juice expressed from the rinds by using the dip type conductivity cell previously mentioned. The variability of the results was still too great to allow any conclusions except of the most general sort. The total range of resistance of the rind juices, as recorded from about 200 determinations, made with the same cell at 22° C., was 150 to 515 ohms. The range in any one storage series was usually less than 50 ohms. The trends shown by the data are presented below.

(1) The earlier determinations seemed to show that the lower the storage temperature, the greater was the conductivity, but so many exceptions were later found that this conclusion did not appear to be justified.

(2) The difference in conductivity between stem-and blossom-end rinds was very slight. The total average resistance of all such determinations of the rinds of field samples taken for immediate study gave 339 and 330 ohms for the stem and blossom ends, respectively. Thus, there was a doubtful tendency toward higher conductivity at

the blossom end. When the results of corresponding determinations on the storage samples were examined, the situation regarding stem and blossom ends was found to be reversed; that is to say, the stem-end rinds showed the higher conductivity.

(3) In all instances recorded the flavedo showed a higher conductivity than the albedo, but the difference was less in the storage samples. This situation may help to explain the apparent reversal of conductivity of the stem- and blossom-end rinds in storage, since the stem-end rind had a larger proportion of albedo.

Pectin Determinations

In order to determine whether there is any relation between grapefruit spotting and the condition of the pectic substances of the rind, a preliminary set of determinations was made on fruit which had been held in storage.

The method used was developed from that given by Carré (*3*), and Carré and Haynes (*4*), and modified by Haller (*10*), Rosa (*21*), and Conrad (*6*).

Fruit was collected on June 15, 1934, from previously selected trees and divided into three equal lots for storage at 36°, 42°, and 52° F. It was removed on July 24 for the determination of pectic substances. At that time all the fruit at 36° was severely spotted; all at 42° was slightly spotted; and all at 52° was sound. The pectic contents of the stem-end rind are given in table 12.

Table 12.—*Pectic content of the Flavedo and albedo of rinds of Marsh grapefruit grown at Claremont, Calif., and stored 39 days at different temperatures*

[Figures are in percentage calculated as calcium pectate on fresh-weight basis]

Substance determined	36° F.		42° F.		52° F.	
	Flavedo	Albedo	Flavedo	Albedo	Flavedo	Albedo
Soluble pectic acid.....Percent	0.0	0.0	0.0	0.0	0.0	0.0
Soluble pectin.....do	.24	.72	.40	.84	.28	.68
Insoluble pectic acid.....do	0	0	0	0	0	0
Protopectin.....do	2.31	1.71	2.56	1.90	2.44	2.01
Total.....do	2.55	2.43	2.96	2.74	2.72	2.69
Protopectin : pectin.....ratio	9.6	2.4	6.4	2.3	8.7	3.0

Soluble pectin was always higher in the albedo, and protopectin in the flavedo, the total pectic content being slightly higher in the flavedo. No pectic acid, either soluble or insoluble, was found in any sample. The ratio of protopectin to pectin was always higher in the flavedo than in the albedo, the average being 8.2 and 2.6, respectively.

There was no correlation between pectin content and temperature, on the one hand, and spotting of the fruit on the other. However, the soluble pectin content of the fruit held at 42° F. was higher, both in the flavedo and the albedo, than in that held at the other temperatures; this brought about a lowering of the ratio of protopectin to pectin (in the combined flavedo and albedo) to an average of 4.4 at 42° as compared with 6 and 5.9 at 36° and 52°, respectively. The

most serious spotting has generally occurred at 42°. More work needs to be done to determine whether there is any definite relationship between spotting and the condition of the pectic contents of the rind.

Buffer System

The frequent tendency of the rind of grapefruit stored at 42° F. to be higher in active acidity than that stored at a higher or lower temperature suggested the desirability of determining whether the different temperatures had different effects on the buffer system.

The fruit used for this experiment was collected at Claremont on June 15, 1934, and various lots were stored at 36°, 42°, and 52° F. At the end of 5 weeks all the fruit at 36° was severely spotted, that at 42° was slightly spotted, and that at 52° was sound. Only the stem-end rind was used.

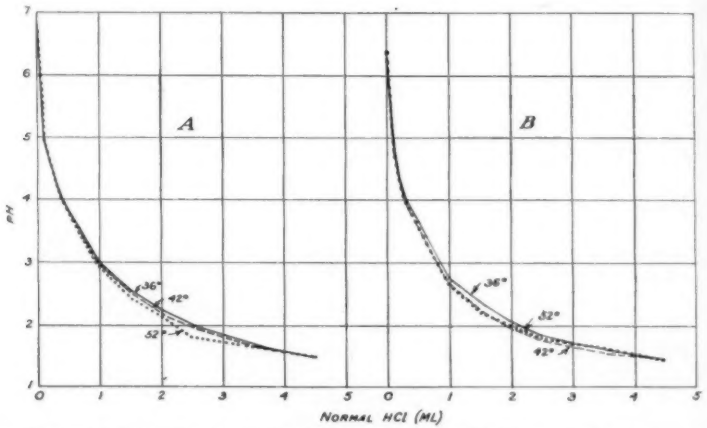


Figure 13.—pH of the flavedo (*A*) and the albedo (*B*) of the rinds of Marsh grapefruit, from Claremont, Calif., after 5 weeks' storage at 36°, 42°, and 52° F., after addition of various quantities of normal HCl. Ten grams of freshly ground tissue in 50 ml of water was titrated with normal HCl as indicated.

The method used was as follows: The fruit was peeled and the flavedo and albedo were separated. The rind was ground in a food chopper equipped with a nut-butter attachment. A 10-g sample was weighed out and 50 ml of water added and the mixture stirred well. Quinhydrone was then added and the mixture stirred again by means of a mechanical stirrer for 1.5 minutes before the initial reading on the potentiometer was made. The stirring apparatus consisted of two parts, one a glass paddle immersed in the liquid and constantly turning and the other a small turntable upon which the beaker was placed. In this manner the entire contents of the beaker were kept in continuous motion while the acid or base was being added and the readings were being made. The paddle and turntable were turned by means of a small electric motor. The high viscosity of the material used necessitated such an arrangement in order to get a uniform mixing of the acid or base after each addition.

Immediately after the first reading was taken 0.1 ml of normal acid or base was added. The whole was allowed to mix 1.5 minutes

before determining the resulting pH. This procedure was repeated with the base till a pH value of about 7 was attained. When acid was used, 0.1=ml portions were added till 0.5 ml had been added, after which 0.5-ml portions were added and later 1.0-ml portions, until about pH 1.5 was reached.

In order to attain pH 1.5 it was necessary to add about 4.5 ml of normal hydrochloric acid to both the flavedo and albedo from all the temperatures. After this amount of acid had been added the pH of the flavedo was 1.50 in rind from the 36° F. storage, 1.51 in that from the 42°, and 1.50 in that from the 52°. The corresponding pH values of the albedo were 1.44, 1.43, and 1.42, respectively. This shows no change in the buffer system brought about by differences in storage temperatures. The titration curves are given in figure 13.

A rapid drop in pH occurred on the addition of the first few tenths of a milliliter of normal acid. In order to see whether there might be differences in this range too small to be observed with the concentration of acid used, another series of titrations was carried through. One-tenth normal hydrochloric acid was used and the same quantities per reading were added as before. Only the albedo was tested. No important differences were observed between the rinds held at the different temperatures. During the addition of the first 0.6 ml the pH of the three rinds dropped at the same rate (fig. 14). From then until 1 ml had been added the drop in the pH of the rind stored at 36° F. was most rapid, next in that held at 52°, and smallest in that held at 42°, indicating the strongest buffer in the material from 42°. At this time the pH attained was 4.78, 4.85, and 4.93, respectively. These relative positions were maintained with gradually diminishing differences until 10 ml had been added, when the pH values were 2.91, 2.91, and 2.94, respectively. At no time was there any indication of a destruction of any part of the buffer system during storage at a temperature of 42°. The titration curves with 0.1 normal acid are shown in figure 14.

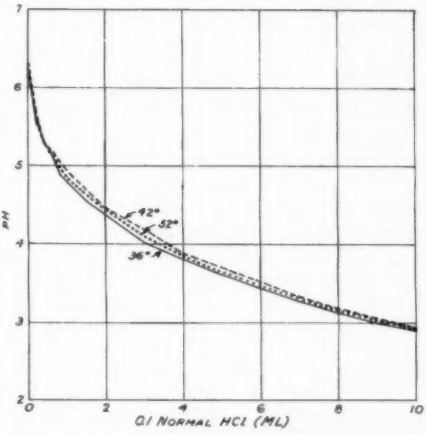


FIGURE 14.—pH of the albedo of the rinds of Marsh grapefruit from Claremont, Calif., after 5 weeks' storage at 36°, 42°, and 52° F., after addition of various quantities of N/10 HCl. Ten grams of freshly ground tissue in 50 ml of water was titrated with N/10 HCl as indicated.

A repetition of this experiment with fruit from Corona and Fontana held at the same temperatures gave similar results. However, with the Corona and Fontana fruit an average of 3.8 ml normal acid was required to bring 10 g of the albedo to pH 1.5, and 5.6 ml for the flavedo. This compares with 4.5 ml for each flavedo and albedo sample from the Claremont fruit.

Injection Experiments

General

Tests were made to determine the effects of certain substances, principally naringin and naringenin, when injected into living grapefruit rinds.

On October 23, 1933, a few grapefruit were set aside from a storage lot which was being preserved for chemical analysis. A 0.5-percent solution of naringenin, the phenolic derivative of naringin, was made up in 5-percent alcohol. Each grapefruit was given three injections of 1 ml of this solution by means of a hypodermic needle thrust diagonally beneath the flavedo layer. The injected fruit was left in the laboratory for 24 hours. On dissection, some of the fruit showed a very slight discoloration in the vicinity of the injection. The remainder of the fruit was taken to a cold room and left at −15° F. for 24 hours, and then brought back into the laboratory and thawed rapidly. When the injected regions were cut across, a pink color was noticed. From time to time it had been noted that the albedo of fruit which had been held in storage at 42° often showed considerable pink color after being frozen in the hardening room in preparation for pH determinations. This pink color had not been noticed at 32° or 52° and its appearance now in association with naringenin was considered very interesting.

In a second injection experiment the following solutions were used: (1) 1-percent solution of naringin in 5-percent alcohol; (2) 1-percent solution of naringenin in 5-percent alcohol; and (3) 5 percent of alcohol in water. The fruit was from Corona and Fontana and had been kept for 4 weeks at 52°, 42°, and 32° F. There were four fruits of each lot and each fruit was injected with 1 ml of each of the three solutions named. After injection they were left in the laboratory for 40 hours and then frozen for 24 hours at −15°.

An examination of the fruits after they had thawed showed no coloration around any of the injections in the 32° F. lots. All of the 42° lots showed a pink color around the naringenin injection. At 52° the Fontana lot showed one case of pink color for the naringenin solution. No other solutions produced the pink color.

On November 20, 1933, 27 grapefruit freshly picked at Oasis were injected and stored at 32°, 42°, and 52° F. for 12 days. The solutions used were 0.1 percent of naringin, 0.1 percent of naringenin in 5-percent alcohol, and 5-percent of alcohol as a control. Injury was evident earlier in the lots kept at 32° and 42°. By the close of the period all naringenin injections at all temperatures had caused serious injury in the form of discolored depressions 1 to 2.5 cm across. There were four traces of injury from 27 injections of the naringin solution but none with the control 5-percent alcohol solution. In general, the injuries were most severe at 42°.

A fourth injection experiment was started on January 20, 1934, with fruit that had been freshly picked at Claremont. Each fruit was injected with 2 ml of each of five solutions, as follows: (1) Control, 5-percent of alcohol; (2) 0.1 percent of naringin in 5-percent alcohol; (3) 0.1 percent of naringenin in 5-percent alcohol; (4) 0.028 percent of naringenin in distilled water; and (5) ferric chloride solution (2 drops of 50-percent solution to 100 ml of water).

Within 4 days the fruits injected with solution no. 3 showed injury at all three storage temperatures and solution no. 5 had caused dark depressions. An inspection on January 29 gave the following results: For solution no. 1, no injury evident; solution no. 2, no injury (all injections appeared exactly like the controls); solution no. 3, depressions and other evidences of injury around every injection (depressions smaller at 32° F.); solution no. 4, some injury, particularly lack of firmness around injections; and solution no. 5, darkening present in all cases. The darkening seemed to be mostly the characteristic reaction of ferric chloride with the naringin occurring naturally within the tissue.

The appearance of the injected areas is shown in plate 2, *A*, *B*. *A* is a photograph of two fruits taken at random from the injected lots shortly after inspection and removal from storage. The 0.1-percent naringenin injections are facing forward in each case. The 0.1-percent naringin injections are to the left, and the 0.028-percent naringenin injections are to the right. The crayon circles on the fruits were in different colors to aid identification of solutions. Plate 2, *B*, is a photograph of the same fruit shown in *A* but taken after the fruit had remained at room temperature for 24 hours. The photograph shows how the naringenin depressions had darkened and become more severe.

A fifth injection experiment was planned to test further the effects of naringenin solutions of various strengths. The fruit used was from Claremont and had been in storage at 32°, 42°, and 52° F. for 26 days. Five fruits were selected for each experiment, and each fruit was injected with 2 ml of the following solutions: (1) Check, 5-percent alcohol; (2) 0.1 percent of naringenin in 5-percent alcohol; (3) 0.05 percent of naringenin in 5-percent alcohol; (4) 0.01 percent of naringenin in 5-percent alcohol; (5) 0.005 percent of naringenin in 5-percent alcohol. After injection the fruit was returned to the respective storage rooms. Inspection in 2 weeks gave the following results: At 32° all five fruits injected with solution no. 3 showed sunken and darkened areas and two injected with solution no. 4 also were injured. All others were sound. Numerically the effects were the same at 42° and 52°, but the degree of injury was greatest at 32° and about the same at 42° and 52°.

A sixth experimental injection was made on fruit selected from the same trees as for the fifth experiment. In the sixth experiment, however, the fruit was injected on the day of picking instead of after a period in storage. The injections were like those in the fifth experiment, and after injection the fruits were placed at the three temperatures previously used. Inspection was made after 2 weeks. At 32° F. all five fruits injected with solution no. 3 were badly affected, as were two of those injected with solution no. 4. No others were affected. At 52° there was no injury from solution no. 4. At 42° the results were numerically the same as at 32°. The relative severity of injury at the different temperatures was in decreasing order at 42°, 32°, and 52°.

SPECIFIC TESTS

In the injection experiments described above no attempt was made to keep the fruit from the different trees separate. The fruit from each picking was placed in one general lot before injection, the divi-

sion being made later for storage at different temperatures. It was noted that certain fruit in the injected lots escaped, or almost escaped, being affected by the naringenin in solutions. Because of the possibility that these manifest differences in susceptibility might be partly the result of differences in individual trees, all further injection tests were made with fruit of definitely known origin.

On February 16, 1934, 15 fruits from each of the 3 trees at Claremont were injected with 0.05-percent, 0.025-percent, and 0.01-percent naringenin solutions. After injection the lots were separated and placed at the three usual storage temperatures. In 4 days inspection indicated (1) that injury was most abundant and severe at 42° F., and (2) that the fruit from tree no. 2 was least injured and that from tree no. 3 most injured.

On February 27 fruit was collected from the same three trees at Claremont. One set was used for the injection test and another for storage at the three temperatures. The injection solutions were 0.03 and 0.015 percent of naringenin. After 5 days' storage, inspection showed that the injuries around the injections were most severe at 42° F. and that the fruit from tree no. 2 showed least and that from tree no. 3 most injury.

The uninjected lots which had been placed in storage were inspected for storage spots about 5 weeks later. There was only a trace of spotting at 52° F., but all fruits at 42° and 32° were slightly spotted. The least severe spotting at the last temperatures occurred on the fruit from tree no. 2.

These injection and storage tests were repeated on fruit from the same trees four more times, on March 19, March 31, May 5, and May 18, and each injected lot was held for 5 days at 42° F. The injection tests in every case showed least injury to fruit from tree no. 2 and most injury to fruit from tree no. 3. In the storage tests the fruit from tree no. 2 always showed the least severe spotting, but that from trees nos. 1 and 3 alternated their order in being most severely affected.

In the tests started March 19 and March 31, fruit from 7 additional trees was collected and injected, together with that from the original 3 trees, and held for 5 days at 42° F. In the first test with fruit from these 10 trees, the three lots least affected by the injections were from trees 2, 4, and 6; those most affected were from trees 9, 8, and 10. In the second test the lots least affected were from trees 4, 5, and 6, and those most affected were from trees 9, 1, and 10.

Similar injection experiments were conducted at about the same time with fruit from selected trees at Oasis. The results need not be described in detail, since they were in general similar to those secured from the experiments with fruit from Claremont. Both series of tests showed that susceptibility to injury from injections of naringenin varied from tree to tree, but persisted in the same relative degree in fruit from the same tree throughout the period of the tests.

DISCUSSION

The results of all the injection tests with grapefruit show that naringenin is extremely toxic to rind tissue. Except in the early tests the strongest solution used was 0.03 percent, but even the 0.015-percent solution was much stronger than was necessary for

the most susceptible fruit. When the 0.015-percent solution was used, each injection consisted of only 0.3 mg of naringenin. This amount when injected into the albedo produced sunken and discolored areas 2 cm or more in diameter. Slight injury was caused by 0.05 mg of naringenin. On the other hand, naringin was only slightly toxic to rind tissue. Thirty to fifty milligrams of naringin have been injected into grapefruit rinds without injuring them. The injuries caused by naringenin are frequently very similar to the common storage spot, both in shape and color, as plate 2, *C*, shows. These fruits had been injected with 0.6 and 0.3 mg of naringenin 2 weeks earlier and held at 42° F. Discolored spots were produced by both quantities of naringenin.

The conclusion is not drawn, from the effect of naringenin on rinds recorded above, that this substance must be the principal cause of certain storage spots; it does seem very probable, however, that grave injury to the rind would result from any metabolic disturbance that would either retard the synthesis of naringin from any naringenin present or hinder the rapid disposal of this derivative as freed by hydrolysis. Zoller (*24*), in his study of naringin, pointed out the possible danger of naringenin to the tissue and suggested that certain pink spots might have been caused by it. In the present experiments it has been shown that naringenin is not only very effective in producing the more common sunken and discolored spots, but also that it produces a pink color in the albedo, which appears to be identical with that developing in uninjected rinds held at 42° F. and subsequently frozen.

In general, the naringenin injuries were most severe when the fruit was held at 42° F. for a few days after injection. It is an interesting fact that naringenin was most effective in causing spotting at temperatures that give the greatest amount of common storage spotting. If an extremely delicate and specific microchemical test for naringenin were available some relation between this substance and storage spotting might be established. So far all the microchemical reactions of naringin and naringenin have been found to be the same.

Other considerations arise from the results of the specific injection experiment in which the fruit of each tree was treated separately. There was a difference in susceptibility to naringenin injury in different fruits and there also seemed to be some consistency in the apparent susceptibility of fruit from different trees. In some instances this susceptibility to naringenin injury seemed to be correlated with the amount of storage spotting of fruit from the same trees. The susceptibility to naringenin injury seemed to vary with the stage of maturity in somewhat the same way as the tendency to spot in storage.

SUMMARY

Results are presented of two series of studies of the changes that take place in the rind of Marsh grapefruit, one in the field, the other in storage. One of the field studies was carried out during the summer of 1933 in the general district that includes Fontana and Corona, Calif., and the other during the winter of 1933–34 in a single locality near Oasis, Calif. These localities represent two important climatic districts concerned in the production of grapefruit.

Seasonal changes in the chemical composition of the rind from the three localities were as follows: (1) Water content showed a slight seasonal trend upward in all three localities. (2) The flavedo-albedo ratio at Fontana and Corona increased slowly to July 23 and then fell below the values found at the beginning of the season. The average ratios were 0.71 and 0.77, respectively. At Oasis the ratio fluctuated with weather conditions, the average being 0.81. (3) Soluble solids increased throughout the season in all three localities, except for a slight falling off at the last collection at Fontana and Corona. (4) Total sugar at Fontana and Corona increased steadily to July 23, then decreased; the graphs for total sugar paralleled those for mean air temperature. At Oasis total sugar tended to increase through the season. (5) Invert sugar at Fontana and Corona increased to July 23, then decreased. At Oasis it did not change significantly until after the third collection, when it increased. The invert-sugar graph paralleled the mean air-temperature graph at Oasis and at Fontana and Corona. (6) The graphs for reducing sugar at Fontana, Corona, and Oasis were somewhat the inverse of the mean-temperature graphs. (7) Naringin decreased in general through the season at all localities, but there were fluctuations due to local conditions. (8) Hydrolyzable polysaccharides showed a steady relative decrease through the season at all localities. (9) Hydrogen-ion concentration of the rind showed only a slight seasonal drop at all localities and that of the pulp juice changed still less.

When the stem-end and blossom-end rinds were compared the following differences were found: (1) At Fontana and Corona the stem-end rind had more water, reducing sugar, invert sugar, and soluble solids than did the blossom end. The latter was higher in hydrolyzable polysaccharides and hydrogen-ion concentration. Naringin and the flavedo-albedo ratio were practically the same in both ends. (2) At Oasis the average differences between the stem ends and blossom ends were very slight except in invert sugar, in which the blossom-end value was significantly higher.

In a comparison of the flavedo and albedo it was shown that (1) the differences between flavedo and albedo tissues were smaller in the grapefruit rind than in the orange rind; (2) the differences were smaller at Fontana and Corona than at Oasis; and (3) the flavedo was higher than the albedo in invert sugar, total sugar, and soluble solids, whereas the albedo was higher in naringin, hydrolyzable polysaccharides, and hydrogen-ion concentration.

The Fontana and Corona rinds agreed more closely in composition than either agreed with the Oasis rinds. The Corona rinds showed a somewhat closer resemblance to the Oasis rinds than did the Fontana rinds.

The main storage experiments were conducted on fruit from Corona, Fontana, and Oasis, but the results of a number of miscellaneous storage experiments on grapefruit rinds are also presented.

A study of pitting and spotting in relation to storage showed:

(1) Fruit from all three localities pitted and spotted most severely at 42° F. and least at 52°, with 32° intermediate.

(2) Mature fruit picked at Corona and Fontana early in the harvest period was more severely affected at 42° F. than that picked in the latter part of the harvest period; at 32° this pitting and spotting

was the reverse of that at 42°; at 52° there was never more than a trace of storage blemishes.

(3) The fruit from Oasis varied strikingly with the season in its susceptibility to pitting and spotting, when held at 32° and 42° F. No pitting or spotting occurred at 52°; but at 32° it increased as the season advanced. At 42° pitting and spotting reached their maximum early in the season, dropped to a minimum in midseason, and increased again toward the end of the harvest season.

Chemical analyses of the rinds of Corona and Fontana grapefruit showed:

(1) Water content decreased slightly at all temperatures.

(2) Total sugar decreased at all temperatures but least at 32° F. and most at 52°.

(3) Reducing sugar decreased slightly at 32° F. but increased at the higher temperatures.

(4) Invert sugar decreased at all temperatures. The decrease was slight at 32° F., but striking and about the same at 42° and 52°.

(5) Naringin usually decreased. The decrease was greatest at 32° F. and least at 52°.

(6) Hydrogen-ion concentration increased at all temperatures. The increase was greatest at 42° F.

(7) There was a high positive correlation between hydrogen-ion concentration and naringin content.

(8) Hydrolyzable polysaccharides increased at all temperatures.

(9) Soluble solids decreased slightly at all temperatures.

(10) Rinds of the fruit from Corona and Fontana behaved essentially alike in all the reactions noted.

Chemical analyses of the rinds of the Oasis fruit showed:

(1) A slight increase in water at 52° F. and a slight decrease at 42° and 32°.

(2) Total sugar decreased at 42° F. and 52°, but increased slightly at 32°.

(3) Reducing sugar increased slightly at all temperatures.

(4) Invert sugar behaved almost exactly as in the Corona and Fontana rinds in all storage collections.

(5) Hydrogen-ion concentration increased in storage at all temperatures but least at 32° F.

(6) Naringin increased at all temperatures, most at 32° F. and least at 52°. The total naringin content showed a high positive correlation with hydrogen-ion concentration.

(7) Hydrolyzable polysaccharides and soluble solids changed only slightly.

A comparison of the Oasis and Corona-Fontana grapefruit rinds showed:

(1) Differences in prestorage composition.

(2) Naringin was hydrolyzed in storage in the Corona-Fontana rinds and synthesized in the Oasis rinds.

Miscellaneous storage experiments showed:

(1) Fruit held for one period at 52° F. and then transferred to lower temperatures spotted only slightly; fruit held at either 42° or 32° continued to spot when transferred to 52°.

(2) Weakly acidified atmospheres accelerated and enhanced pitting and spotting, whereas weak alcohol and ammonia seemed to retard it.

Alcohol also retarded the development of yellow color in the fruit in storage.

(3) The permeability of grapefruit rinds to their own soluble substances increased as the storage temperature was lowered.

(4) The conductivity of the flavedo was higher than that of the albedo, but this difference was reduced during storage. The difference between stem and blossom ends was slight. Variability of conductivity in the same lots of tissue was relatively great.

(5) Soluble pectin was higher in the albedo, and protopectin and total pectic substances were higher in the flavedo. No pectic acid was found in any instance. The soluble pectin content was highest at 42° F. and the protopectin-pectin ratio lowest.

(6) No significant alteration of the buffer system due to storage temperatures was noted.

Injection experiments showed:

(1) When solutions of naringin and its phenolic derivative, naringenin, were injected into the albedo of grapefruit rinds naringenin was approximately 1,000 times more toxic to rind tissue than naringin.

(2) After rinds were injected with 0.1 to 0.6 mg of naringenin and held for 5 days at 42° F., they often showed spots similar to those commonly observed in storage.

(3) If rinds were injected with naringenin and held for a few days at 42° F., then frozen and thawed, the injected areas showed a pink coloration similar to that sometimes observed in stored grapefruit. In rinds injected with dilute naringenin solutions the injury was most aggravated by holding at 42°, which, of the three temperatures used throughout the storage experiments, caused the greatest amount of spotting.

(4) Individual fruits showed different degrees of susceptibility to naringenin injury. Furthermore, the fruit of individual trees seemed to show collectively somewhat consistent relative susceptibility or immunity to injury from given quantities of naringenin. Usually, uninjected fruit from the extreme-case trees mentioned above, when stored for several weeks at 42° F., developed degrees of storage spotting in rather interesting agreement with the susceptibility or immunity to naringenin injury previously noted for them.

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THE HONEYBEE AND THE BEEHIVE IN RELATION TO FIRE BLIGHT [1]

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INTRODUCTION

The chief objective in fire blight control has been the eradication of hold-over cankers, since these cankers are known to be the principal source of primary inoculum in the spring. Consequently the possibility suggested by Rosen (*11*, *12*)[3] that the fire blight organism, *Erwinia amylovora* (Burr.) Bergey et al., may overwinter in the beehive has complicated the problem by introducing one more possible source of infection. Following the appearance of Rosen's first report in 1930, studies were begun in several States to determine the role played by the honeybee in the dissemination of the bacteria and to ascertain whether the bacteria overwinter in the beehive.[4] The findings from New York, Ohio, and California detailed later, which are at variance with those from Arkansas, receive further support from this undertaking, an abstract of which has already been published (*5*).

The fact that the honeybee is an important pollinating agent for fruit blossoms and that individuals from colonies of bees regularly visit flowers at a time when they are highly susceptible to infection by the fire blight organism makes this problem a most important one. It must, however, be remembered that the worker bees which visit blossoms are not those which have had contact with contaminated material the year before, since the life span of the worker is short. Normally no bee would be living in the colony at the time of visits to fruit blossoms which had been there the year before except the queen bee, and queen bees do not visit flowers. There remains, however, the somewhat remote possibility that in some manner the queen bee might be involved in harboring the fire blight organism. Attention must also be directed to other places within the hive where the fire blight bacteria might remain, such as in honey or in other materials in and about the hive. These facts, then, center the investigation largely on the behavior of the bacteria when introduced into the hive, and it is on this point that special emphasis has been placed in this work.

The chief aim of this investigation has been: (1) To determine the longevity of the fire blight organism in the beehive when introduced through the natural channel in the food of the bees, but other aspects of the problem have also received attention, such as (2) the longevity of the bacteria in sugar solutions studied over a wide range of concentrations and temperatures; (3) the location of the bacteria in relation to the life-cycle stages of the insect; and (4) the extent of

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[2] The writers are indebted to Dr. A. W. Woodrow, formerly of the Department of Entomology, for assistance in handling the bees used in these studies.
[3] Reference is made by number (italic) to Literature Cited, p. 809.
[4] Since the environment within the beehive may be assumed to be virtually identical throughout the country the results from the several States should be in close conformity with each other.

dissemination of the bacteria from the beehive to flowers in cages in the orchard.

In the spring of 1932 during the course of observations on the conduct of honeybees visiting pear and apple flowers in the orchard of Cornell University, it was observed that some of the flowers farthest along in development presented a water-soaked appearance. Examination under the microscope showed the flower tissues to be gorged with bacteria. Noting that the bees visited flowers in the clusters containing these water-soaked individuals, a preliminary isolation experiment was made to determine whether the fire blight bacteria were associated with the bees. When the first apple petals were beginning to fall, 10 bees were captured from blossom clusters containing such water-soaked blossoms. At three subsequent biweekly intervals, when blossoms were no longer available, lots of 10 bees each were taken at the entrances to hives in an apiary approximately a half mile away. The fire blight bacteria were obtained from 3 of the 10 bees captured from the blossoms, but no organisms were recovered from the bees subsequently taken in the apiary. In the blight epiphytotic which developed that spring as many as 90 percent of the blossom clusters in Wealthy and 50 percent in McIntosh blighted, providing ideal conditions for this isolation experiment. However, because the bacteria were obtained from the bees only while they were visiting the flowers in the orchard and not thereafter, it appeared that the organisms were incapable of remaining associated with the bees after the blossom period. Accordingly experiments were planned in an attempt to clarify the situation.

LONGEVITY OF THE FIRE BLIGHT BACTERIA IN THE BEEHIVE

PREVIOUS OBSERVATIONS

Although Waite (*20, 21*) was the first to implicate the honeybee with the dissemination of the fire blight organism, it remained for Gossard (*2*) and for Gossard and Walton (*3, 4*) to initiate studies on the longevity of the bacteria in the beehive. In the first report, Gossard (*2*) concluded that the organism of fire blight may live in honey for 47 hours. Later Gossard and Walton (*3*) extended the period of viability in honey to between 72 and 100 hours. In their final report (*4*) they stated that during the blossom period fire blight developed in tender twigs that were inoculated with honey drawn from three different hives. Blight apparently resulted also when apple pollen, removed from the pollen baskets (corbiculae) of bees and the mouth parts of similar bees caught as they were entering the hives were inserted into incisions in tender apple twigs. They further state (*4, p. 86*):

> We were unable to obtain cultures of fire blight from hives in early spring, from either honey or wax and because of its eventual death in honey, we believe it does not exist in the hive at the opening of the season. Dozens of samples were taken and cultured by the plate method in this quest.

The investigations of Gossard and Walton have been extended by a number of investigators. Rosen (*11, p. 302*) stimulated interest in this question when he stated that—

> * * * the writer has successfully isolated the fire-blight pathogen from beehive material gathered throughout the summer, winter and early spring and from the bees themselves obtained from the hives in the early spring prior to the development of blight.

If this statement were applicable elsewhere it would mean that fire blight control cannot be successfully accomplished by canker eradication alone. Thomas (*15*) reported that when the organism causing fire blight was introduced into honey and applied to the surface of honeycomb and wood frame, it was recovered after 15, 55, and 20 days. In Rosen's final report (*12*) covering 5 years' work, he had obtained only 1 positive culture in a total of 1,277 isolation trials from 1931 to 1933, which is in strong contrast to 20 positive isolations in 2,157 trials for 1929 and 1930. The materials used consisted of honey plus comb and bees taken from beehives located in a blighted orchard. The results of a study by Thomas and Ark (*17*) conducted in California did not support Rosen's conclusion. In no instance did they obtain the blight organism from hive material (550 cultures), and they obtained it from bees only when blossoms of fruit trees were available to the insects. Pierstorff and Lamb (*10*) also were unable to demonstrate the presence of the fire blight bacteria on the combs, frames, or in the honey in a beehive 24 hours after a water suspension of the organism had been introduced. They were successful, however, in recovering bacteria from the heads of the honeybees taken from a hive 2 days after it was contaminated with the organism. Parker (*9*) was unable to reisolate the bacteria in the early spring from beehives that were artificially contaminated the preceding November.

MATERIALS AND METHODS

Any study in which honeybees are used for experimental purposes should take into account the habits and condition of the insects. Besides the fruit-blossom period in May, early October is a good time for conducting such a study since the incoming supply of nectar is low in these months and the bees are inclined to stay inside the hive and to concentrate sugar solutions with relative rapidity.

The feeding of honeybees with artificially prepared food containing an abundance of bacteria introduces the bacteria into the hive in a manner comparable to such natural channels as may exist, obviates the necessity for disturbing the working conditions of the bees, insures the entrance of bacteria in great numbers and at the same time facilitates the reisolation of the bacteria at stated intervals after their entrance. These precautions were needed since present isolation methods are not sufficiently selective to be reliable for small numbers of these bacteria. Moreover, this approach takes into account the effect on the nectar or sugar solution produced by the bees in their function of elaborating honey from nectar.

The same two colonies of bees were used throughout the series of tests, which extended over a period of four seasons. Each colony or nucleus hive was fed diluted honey or cane sugar (sucrose) solution of 60 percent sugar concentration in the autumn, or 50 percent concentration when fed in the spring, to which vast numbers of the fire blight bacteria had been added.

The sugar solutions were prepared under approximately aseptic conditions in the same way for each of the six feeding experiments involving hives of bees. Approximately 5 gallons of the prepared solutions were fed to the respective colonies in the fall and 1 quart to

the nucleus hives [5] used in the spring. The sucrose solution was sterilized at 15 pounds pressure for 20 minutes. The diluted honey was brought to a boil. When cool, the bacteria in water suspension were added. The individual bacteria were thoroughly distributed in suspension by careful stirring in the thinner solutions so that in subsequent concentration of these solutions by the bees, there was ample opportunity for the solutions to act in whatever manner they would on the organisms. These precautions were taken in order to obviate the possibility that groupings or clumps of the organisms may protect some bacteria from the action of the concentrated solutions (honey) and thus increase their longevity.

The bacteria for furnishing the inoculum were varied. In 1932, 1 culture from California was employed; in 1933 a composite of 30 cultures; in 1934 a composite of 4 cultures; and in 1935 the progeny of a single-cell culture from New York. The numbers of bacteria per cubic centimeter of solution fed in series 1932a and 1933a were computed by means of the Petroff-Hausser direct bacteria counter and were, respectively, 192 and 155 millions. In the other series the numbers of bacteria were made comparable by means of the McFarland nephelometer (7).

The inoculum except when stated otherwise consisted of a 2-day-old growth on nutrient agar.

The isolation method used consisted of: (1) The transfer of relatively uniform quantities of material with a 5-mm loop to nutrient-dextrose broth, and (2) the subsequent transfer to nutrient agar when growth was evident. The culture-plate method used in the preliminary studies was later abandoned. All the cultures obtained during summer, autumn, and winter that simulated the fire blight organism were inoculated into green Kieffer pear fruits. Shoots on pear and apple trees grown in the greenhouse were used for making pathogenicity tests in the spring when fruits were not available. The surface of fruits was sterilized with alcohol and after inoculation incubated in moist chambers at 24° C.

In pursuing the investigation on the longevity of the fire blight organism in the beehive, a series of six isolation experiments were conducted in October and May. These are designated 1932a, 1933s, 1933a, 1934s, 1934a, and 1935s.[6]

AUTUMN TRIALS

Experiment 1932a

In experiment 1932a, the fire blight bacteria disappeared within 1 week after the close of the feeding period from honey elaborated from food containing the organisms. The bacteria had likewise disappeared from (1) the wax of the combs, (2) the surface of wooden frames, and (3) the mouth parts, alimentary tracts, and honey stomachs of the bees. A summary of this experiment appears in table 1.

[5] "Nucleus hive" is a term employed by beekeepers for small hives used in mating queen bees. These small hives were used because the full colonies were too large to be included in the cages employed.

[6] The symbols s and a signify, respectively, spring and autumn trials.

TABLE 1.—*Summary of isolation studies to determine the longevity of Erwinia amylovora in the beehive when introduced through the natural channel of foods in spring (s) and autumn (a) trials, 1932–35*

Source	Time interval	1932a [1]		1933s		1933a		1934s		1934a		1935s	
		Trials	Positive	Trials	Positive	Trials	Positive	Trials	Positive	Trials	Positive	Trials	Positive
	Days	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*
Honey	1					45	20.0			30	33.3		
	2					40	10.0			30	6.7		
	3					40	.0			30	6.7		
	Over 3	100	0.0	30	0.0	320	.0	30	0.0	90	.0	30	0.0
Wax	1					20	.0			15	13.3		
	Over 1	50	.0	30	.0	200	.0	30	.0	70	.0	30	.0
Wood	1					20	.0			15	.0		
	Over 1	50	.0	30	.0	200	[3] 5	30	.0	70	.0	30	.0
Bees	1					100	6.0			85	11.8		
	Over 1	50	.0	30	.0	50	.0	30	.0	50	.0	30	.0
Pollen	Over 5			30	[3] 3.3			30	.0			30	.0

[1] Isolation interval=7 days.
[2] Isolation interval=12 days.
[3] Isolation interval=13 days.

Two colonies of bees were used. One received contaminated sucrose solution and the other contaminated diluted honey prepared as stated earlier. "Atmospheric" tin-pail feeders with perforated tops were filled with the solutions and inverted, being placed in empty hive bodies over the hives. Except at daily or 2-day intervals when the food supply was replenished, the bees were not disturbed. The feeding was begun on October 6 and the last food was consumed on October 19. An extensive isolation experiment was arranged in which samples of the honey in the comb elaborated respectively from the honey and sugar solutions were incubated in previously sterilized specimen dishes over a range of temperatures varying at 3° intervals from 3° to 24° C.

Since earlier investigators in this field had found that the fire blight organism survives for considerable periods within the hive, the first isolations were not attempted until 7 days after the close of the feeding period. At the first isolation interval the materials were taken from the hives and placed in the dishes in the incubators. From each hive they consisted of 10 transfers of honey and 5 transfers from comb cappings and side walls of cells and scrapings from the frames. At the same time five bees were collected from each hive. From each bee the mouth parts, honey stomach, and alimentary tract were cultured separately. None of the sources tested yielded the bacteria, but the pathogen was readily obtained in all but the last case from samples of the original sugar and honey solutions that had been incubated at 6°, 12°, 18°, and 24° C. Moreover, during the course of the feeding period, the bacteria were successfully recovered at daily intervals up through the twelfth day from the original solutions stored in a shed at approximately 18°, indicating that the pathogenic bacteria survived in the food during that period.

At four subsequent weekly intervals similar isolation series were repeated, always with negative results. At the termination of this experiment, pathogenic bacteria were still being recovered from the original solutions held in the 6° and 12° C. incubators. In these studies the wax, wood, and bee samples were taken from the respective hives at the times indicated. The "honey" at the later weekly intervals was taken from the samples placed in the 24° incubator. These results are interpreted as meaning that the bacteria cannot survive the operations performed by the bees, but precise information was not obtained on the rate of disappearance of the bacteria in the operation of concentrating the food supply. The bacteria apparently had died at a more rapid rate than was expected, necessitating much shorter isolation intervals for determination of the rate of destruction.

Experiment 1933A

In the fall of 1933 the fire blight bacteria were recovered from honey produced from contaminated sucrose and honey solutions within 2 days from the time they had been taken by the bees.

Feeding was begun on October 5, and by October 16 and 18 the respective dilute honey and sucrose solutions were all removed from the feeders by the bees. At the end of the first day one frame was removed from each of the hives and placed in a 30° C. incubator in the laboratory. Near the middle of the feeding period another frame from each of the respective hives was removed and placed in the incubator. In this experiment 40 attempts to isolate the organism were made from the honey, and 20 from each of the wax and frame surfaces at intervals of 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 18, and 22 days after the bees had taken the contaminated food. Six of the honey isolations made on the first day and six of those made on the second day, or 15 percent of each, yielded the fire blight bacteria. All the others were negative.

Nonpathogenic bacteria were obtained in a large percentage of the isolation tubes of the first six isolation intervals. Their growth in nutrient-dextrose broth was unlike that of the fire blight organism, being much slower and rarely if ever resulting in the formation of a pellicle which is so characteristic of *Erwinia amylovora*. After the first week, when the isolation intervals were lengthened to 2 days, many of the tubes remained sterile and the majority of the others were free of microflora except for occasional molds. All isolations attempted from comb surface for the presence of the fire blight bacteria were negative. Likewise all attempts to recover these bacteria from scrapings of the wood frame were negative with one exception, namely, an isolation attempted on the twelfth day. During the course of this experiment isolations were attempted at the same time intervals from the bees in the two hives. Since the bees were manipulating contaminated food for approximately 2 weeks, a relatively large percentage of the isolations were from bees taken while actually feeding. During this time isolations were tried from the mouth parts, honey stomachs, and alimentary tracts of 100 bees, only 6, or 6 percent, of which yielded the fire blight bacteria. In three instances the bacteria were present both on the mouth parts and in the honey stomachs; in two instances in the alimentary tracts and honey stomachs; and in one instance on the mouth parts alone. Isolations attempted from 10

bees at intervals of 2, 3, 4, 5, and 6 days after the close of the feeding period were all negative. From these data it is apparent that the fire blight organism was not as abundant on the bees as might have been expected. It was further observed that of the positive isolations obtained, there was approximately equal division between the diluted honey and the sucrose solution sources which are considered under one heading in the table.

Experiment 1934a

In the third and final experiment conducted in the fall of the year the fire blight bacteria had disappeared from the honey elaborated from heavily contaminated sucrose solution within 3 days from the time of elaboration by the bees. This experiment, except for a few changes, was similar to experiment 1933a, and the results were in conformity with the previous findings (table 1). The feeding of diluted honey solutions contaminated with the bacteria was omitted because of the uniformity of results of the two solutions in previous trials.

In order to obtain a check on the microflora contributed by the bees and the hive during the concentration of sugar solution the bees were fed sterile sucrose solution for 2 days before they were given the contaminated food. Feeding contaminated food was begun on October 18 and completed on October 25. The number of isolations intervals was reduced, and consisted of 1, 2, 3, 4, 5, and 7 days. The number of isolation trials from the various sources was not changed from the previous corresponding experiment, 1933a. Four series of isolation trials were conducted. The first was confined to the concentrated material from sterile sucrose solution which yielded only molds and miscellaneous bacteria. The other three series were begun on, respectively, October 18, October 23, and October 24. In the first two instances, the frames used in making the isolations were taken from hives and stored in a 30° C. incubator in the laboratory. The third frame was left in the hive and removed only long enough for samples to be taken when it was again replaced. Isolations from the bees were confined to the use of mouth parts and honey stomachs.

Of the 30 isolations made on the first day from the concentrated sucrose solution which had contained fire blight bacteria, 10, or 33.3 percent, were positive for the fire blight organism. From similar numbers of isolation trials on the 2 successive days, there were for each interval two positive, or 6.7 percent. All the later attempts to recover the fire blight bacteria from the concentrated sucrose solution were unsuccessful.

The bacteria were recovered in two cases from bits of wax taken from the combs at the first interval of 1 day, or in 13.3 percent of the attempts. All 70 subsequent trials for the isolation of the bacteria from this source in this experiment were negative. No fire blight bacteria were recovered from 85 samplings of scrapings removed by a sterile scalpel from various parts of the frame.

The bacteria were likewise recovered from 10 of a total of 85 bees, or in 11.8 percent of all attempted isolations made during the time that they were working with the contaminated food. All attempts to recover the bacteria from bees separated from the contaminated feed by more than 1 day were negative. The location of the bacteria in

these bees was chiefly in the honey stomachs, since recovery was made from this source eight times to the three that they were obtained from the mouth parts. From these experiments it is evident that the fire blight bacteria are able to survive in the beehive for a relatively short time.

SPRING TRIALS

EXPERIMENT 1933s

This study was conducted in the spring of 1933 in the orchard of Cornell University with special cages, which are described later. The honeybees were confined with apple blossoms of the Rhode Island Greening variety. A nucleus beehive was outfitted with two feeders, one containing contaminated sucrose solution and the other contaminated honey solution, one frame of brood ready to emerge, one empty comb, and two cupfuls of worker bees. No queen was included. The nucleus hive was assembled the afternoon of May 13 and installed in the cage in the evening. The hive entrance was opened after dark. During the forenoon of the same day, with the flowers in the late pink stage of development, a lime-sulphur spray (1–40) was applied and in the afternoon an application of nicotine sulphate (1–800), in order to remove insects that might cause complications. Daily observations were made and a record was kept of the behavior of the bees.

A few flowers had begun to open on May 14. At 9 a. m. May 15, the flowers were in early bloom and counts were made of the number of bee visits to groups of 10 blossom clusters in four different cages. For 5-minute intervals there were, respectively, 5, 1, 3, and 4 visits made by the bees, and only an occasional bee was observed trying to gain freedom by flying against the sides of the cage. The flowers came into flull bloom on May 17 and the bees were observed freely visiting them up through May 20 at petal fall. The food was all converted into "honey" approximately 5 days before the cages were removed, and the hives were examined on May 26. Immediately after the cages were removed attempts were made to recover the bacteria from the hives and the bees, and also from apple pollen. The first isolations were made 13 days after the beginning of the experiment and approximately 5 days after the feeders were emptied. The results of these isolation trials were all negative but for one instance (table 1) in which the bacteria were recovered from pollen taken from a cell of pollen. Two subsequent series of isolation were made at intervals of 20 and 27 days from the time of starting the experiment, but the results were all negative. The controls, consisting of the contaminated solutions held at 6°, 12°, 18°, and 24° C., all yielded the bacteria at the end of 13 days except the last.

These data support the findings of the autumn experiments, and indicate that the fire-blight bacteria disappear within 5 days from the time contaminated food is converted into honey.

EXPERIMENT 1934s

In experiment 1934s, which was a repetition of 1933s, negative results were obtained in all cases for the recovery of the blight bacteria from the nucleus hive. The Twenty Ounce apple variety was here substituted for Rhode Island Greening.

Using the same general procedure as before, the hives were assembled and installed in the cages in the orchard on May 18 and were removed on May 29. On the latter date, isolations were attempted from the same sources as before at approximately 4 days after the last of the food was taken. The fire blight bacteria were not recovered. Two similar isolation series were made at successive 2-day intervals after the first attempts, but no fire blight bacteria were obtained.

Experiment 1935s

The final experiment, which was conducted in the spring of 1935, was a repetition of experiment 1934s.

Bees were placed in the hives on May 21 and the cages were installed on May 22. On June 3, 12 days later, the cages were removed and isolations were tried from the usual sources (table 1). The food had all been consumed approximately 4 days previously. The first isolations attempted were all negative for the fire blight bacteria. Subsequent attempts gave similar results. Isolations from a sample of the infested original contaminated sucrose solution stored at shed temperature (18° C.) yielded pathogenic bacteria to the close of the experiment. The rate of disappearance of the bacteria was not accurately determined in the experiments conducted in the spring of the year because a disturbance to the bees would have altered the experiment in other respects.

Discussion

The consistency of the results in all these experiments conducted in the spring and in the fall, in which the bacteria disappeared from the beehive, has an important bearing on the question of the possible role of the bees and the beehive in the dissemination of the fire blight organism.

The rapid disappearance, usually within 2 days, of fire blight bacteria after their introduction into the hive attests to the destructive properties of honey and of the beehive environment, so far as fire blight bacteria are concerned. The two minor exceptions reported above would not appear to invalidate this conclusion, and even for the longest periods of survival encountered, it will be recalled that they were from pollen and scrapings of the wood, in both of which instances there was little opportunity for any possible disinfecting effects of honey to operate. There is, of course, some honey used in the storage of pollen in cells of the comb. The sterilizing action of honey applies as well to other nonspore-forming bacteria previously studied (*13*) and to the nonspore-forming bacterial flora normally associated with honeybees and the interior of the hive, which survived in honey only a few days longer than the fire blight organism. The relatively short length of life of fire blight bacteria in all the locations studied within the hive probably cannot be ascribed to the sterilizing effects of honey alone or to any other one factor, but is more probably the result of the interaction of a number of factors, including the materials added to sugar solutions by the worker bees in their elaboration of their food supplies. These factors of hive environment consist in part of a high relative humidity (70 percent or even higher during the incoming of fresh food supplies) and of high temperatures (roughly 94° F., within the brood nest of the hive in which most of the elaborated food is stored at least temporarily). Parker (*9*) has

demonstrated the detrimental influence of high relative humidities on the survival of fire blight bacteria, and, as will be discussed later, high temperatures, within the range of temperatures found within the hive, are also detrimental to their survival. Whether the two factors of destructive relative humidities and high temperatures fully explain the rapid death of the fire blight bacteria within the hive but outside of honey is somewhat questionable, but that they contribute to this end appears evident.

The bees used in these experiments together with the honey they produced were used in cage experiments in the orchard to check further the possibility of the bacteria overwintering in association with the bees that had elaborated the contaminated food.

EFFECT OF SUGAR SOLUTIONS ON THE FIRE BLIGHT ORGANISM

A number of workers have studied the effect of sugar concentration on the fire blight bacteria. Experiments by Gossard and Walton (*4*) demonstrated that the fire blight organism was able to live for about 4 days in honey, for 10 days in aphid honeydew, and for 5 days in peach, plum, and cherry nectar. McLarty (*8*) stated that fire blight bacteria lived for 48 days in strained honey. According to Parker (*9*), McLarty stated in correspondence that he later found the bacteria to live for 84 days in the same honey. Thomas (*15*) reported that the organism causing fire blight lived for 15 days in honey. Using a special nutrient solution as a base, Thomas and Ark (*16*) determined the maximum concentration of sugars that the organism will tolerate in culture solutions. In dextrose solutions very slight growth was obtained at a concentration of 28 percent while optimum growth occurred at 3 percent. In solutions of levulose and sucrose optimum growth was near 10 percent, but the maximum growth concentrations were, respectively, 18 and 58 percent. The fire blight organism was found to survive for several weeks at 60-percent sucrose concentration. They further point out that the concentrations of the sugars which inhibited the organism corresponded approximately with the osmotic values of the sugars. The longevity of the bacteria in both artificial and natural honey was found by Pierstorff and Lamb (*10*) to be 5 days.

There is considerable divergence in the results of these various authors. To some extent this may be explained by differences in the degree of individual dispersion of the bacteria within the solutions, for it is evident that if the bacteria were clumped in some cases increased survival might result. It is further to be noted that none of these authors has stated the temperatures at which he worked, and temperature is an important factor in survival of the fire blight organism in sugar solutions.

Because of this lack of agreement in the work of other investigators, tests were made of the survival of fire blight organisms in various sugar solutions, the sugars used being those which occur in nectar and honey, namely, levulose, dextrose, and sucrose, used singly or in combinations.

LONGEVITY OF BACTERIA IN SUGAR SOLUTIONS

The longevity of the fire blight bacteria was first tested in the fall of 1932 in artificial nectar (21.2-percent sugar) compounded according to the method of Beutler (*1*) from sucrose, levulose, and dextrose, of,

respectively, 8.4, 7.3, and 5.5 parts by weight. The sugar solutions were sterilized by filtration through a Berkefeld filter. For supplying the mineral elements a given volume of honey was ashed and a quantity of it corresponding to the amount in nectar was added aseptically to the synthetic nectar solution. A loopful (6 mm) of a dense water suspension of the fire blight organism was added to the artificial nectar. Samples were incubated in flasks at 3°, 12°, and 21° C. Isolations were made at daily intervals for 1 week and at weekly intervals thereafter for 6 weeks. The bacteria were still alive at all the temperatures except 21° at the end of 6 weeks, when the experiment was terminated.

The longevity of the bacteria was tested simultaneously in pure honey, diluted honey, and sucrose solution held at 6° C. The diluted honey and the sucrose solution were samples of the materials fed the colonies of bees in the 1932a experiment. The bacteria survived in these solutions for 31, 20, and 37 weeks. Apparently the low temperature favored their survival.

At the same time, a third experiment was run to test the longevity of the bacteria in duplicate tubes in different concentrations of sucrose solution held at 3° and 21° C. The concentrations tested were 0, 1, 5, 10, 20, 40, and 60 percent of sucrose in water. At 3° the bacteria survived for 28, 9, 8, 8, 6, 10, and 12 weeks, while at 21° the survival periods were 28, 7, 4, 4, 5, 5, and 1 weeks.

A more extensive experiment was begun in December 1932. The sugars tested included sucrose, dextrose, sucrose+dextrose in equal amounts, levulose, synthetic nectar (according to the findings of Beutler (*1*)), and unsterilized honey. The concentrations used with each material were, respectively, 0, 1, 5, 10, 20, 30, 40, 50, 60, and 75 percent of sugar, each test being made in quadruplicate. The fire blight bacteria were placed in test tubes containing 10 cc of these sugar concentrations held at 6°, 12°, 18°, and 24° C. All except those containing levulose were sterilized in the autoclave for 15 minutes at 15 pounds pressure. To avoid caramelization where levulose was used alone or as a constituent the individual sugars were added to hot sterile distilled water, and heated in the Arnold steamer for 12 minutes on 3 successive days. The inoculum consisted of a highly virulent culture of the organism that had been isolated from crab apple in California. The results of this experiment are contained in figure 1, *A*. Since no special provision was made for retarding evaporation, there was a marked tendency at 18° and 24° for the sugar solutions to become more concentrated. Thus, for example, the 20-percent sucrose solution incubated at 18° in a period of 35 weeks became concentrated to 59.69 percent sugar. To determine this, examination was made with an Abbe refractometer (drop). Similarly, the 10-percent sucrose solution in 36 weeks became concentrated to 51.22 percent. Obviously this fact should be kept in mind when evaluating these data. In sucrose solution the bacteria survived for a relatively short time in concentrations of 40 percent and above, but there was a discrepancy in the effect of temperature on survival in which 18° appeared to be more favorable than 6°, 12°, and 24°. For dextrose the critical concentrations were 10 percent and above. Mixtures of equal parts of sucrose and dextrose acted much the same as dextrose alone. Levulose appeared to be somewhat less destruc-

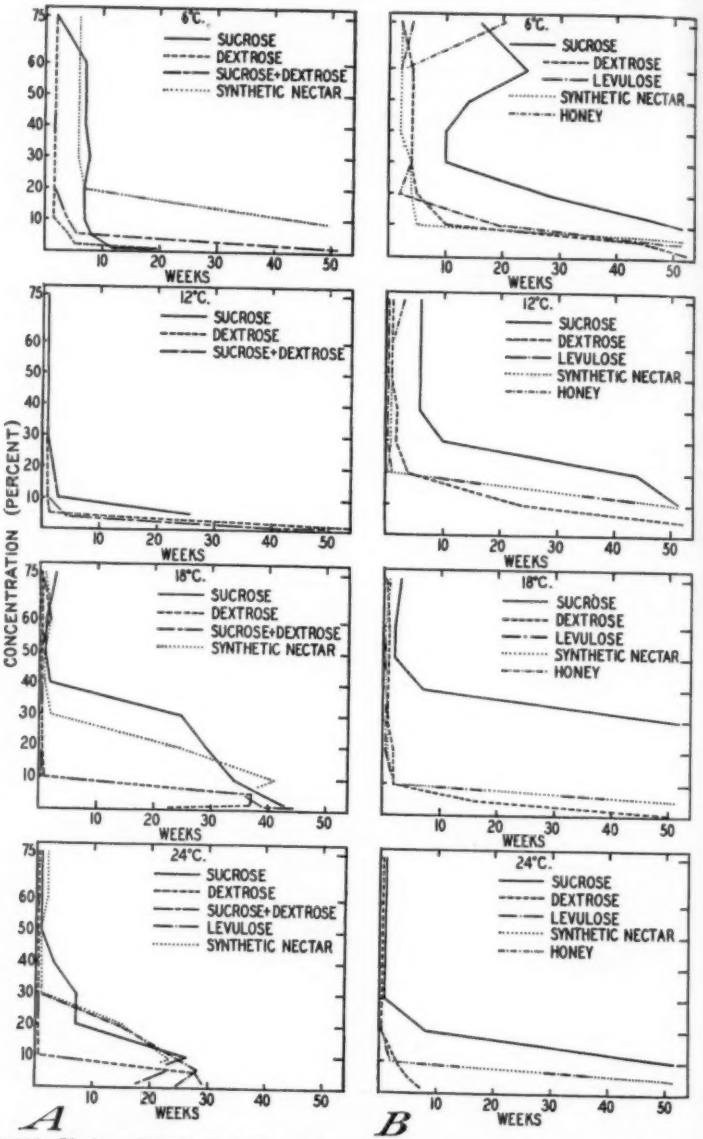

FIGURE 1.—The longevity of the fire blight organism, *Erwinia amylovora*, in solutions of various sugars in different concentrations, and in synthetic nectar and honey held at constant temperatures. The inoculum was varied for the two series. *A* involved a single culture and *B* a composite of 30 cultures. The incubation temperatures were 6°, 12°, 18°, and 24° C.

tive of the bacteria than dextrose, but rather similar to sucrose. Synthetic nectar with and without ash behaved more like levulose than like the other sugars. The addition of ash to the synthetic nectar solution appeared to upset the effect of the sugars alone by decreasing markedly the longevity of the bacteria, especially at the lower concentrations, and therefore was omitted in the next experiment. The higher concentrations of all the solutions behaved similarly in killing the bacteria in relatively short periods of time. The survival of the bacteria in a series of unsterilized honey dilutions comparable to the synthetic nectar was similar to the results in the latter. This unsterilized honey series was abandoned at 6 weeks because of contamination by molds. Survival was usually longest at the lower temperatures, but this was not always the case.

Another experiment to test the longevity of the fire blight organism in sugar solutions was begun in November 1933. Certain modifications were adopted, however. A composite culture of 30 organisms, isolated from widely separated places, was used instead of a single culture. The bacteria were added at the rate of approximately 250 million per cubic centimeter determined by use of Petroff-Hausser direct bacteria counter. The respective sugars, sucrose, levulose, and dextrose, were used alone and in combination simulating nectar (*1*). Extracted honey of approximately 80-percent sugar concentration and the same honey diluted to 60-percent concentration of sugar were also included. No ash was added to the nectar. To reduce evaporation, lead foil was wrapped over the tops of all the tubes held at 18° and 24° C. Before the experiment was begun, the levulose and nectar solutions were heated for 12 minutes in the Arnold steamer on 4 successive days. The sucrose and dextrose solutions were sterilized in the autoclave for 15 minutes at 15 pounds pressure. This experiment was terminated at the end of 1 year; the data obtained are summarized in figure 1, *B*. From figure 1, *B*, it is evident that the results correspond to those already discussed in previous experiments. Sugar solutions at concentrations simulating honey (75 percent) and held at temperatures nearest to those which would obtain in the apiary in the summer (24°) are destructive to the bacteria, ordinarily killing them within 1 week. At temperatures more nearly approximating those of the hives in winter (18°), bacterial survival is not sufficient to account for the hive being a potential overwintering source. No definite explanation can be given for the relatively long survival of the bacteria of the composite culture of 30 strains. However, it may be explained in part by reduced evaporation and consequently a lower concentration of sugar.

GROWTH OF BACTERIA IN SUGAR SOLUTIONS

Studies were next made of the growth of the fire blight organism in a special synthetic medium (*14*) when the carbohydrate sources were the respective sugars. The sugar solutions were sterilized by filtration through a Berkefeld filter. In series 1, duplicate tubes were prepared of each of the sugars (sucrose, levulose, and dextrose, and the synthetic nectar mixture) of sugar concentrations of 0, 2, 5, 10, 15, 20, 30, 35, 40, 45, and 50 percent. In series 2, the concentrations were somewhat modified, but covered the same range. Series 3 involved the use of a number of sugar concentrations that differed from each other in smaller amounts as the concentrations approximated

the critical growth limits of the bacteria. The progeny of a single-cell strain of the fire blight organism grown for 2 days on nutrient agar furnished the inoculum. All cultures were incubated at 24° C. The results obtained are summarized in table 2. These data indicate that the monosaccharides, dextrose, and levulose permit a slight growth in sugar concentrations as high as 30 percent and that in sucrose, a disaccharide, the bacteria made a very small but perceptible growth at the 58-percent concentration. These results are in approximate agreement with those of Thomas and Ark (*16*). When the sugars were combined into a mixture simulating nectar (Beutler (*1*)), the bacteria grew at 35 percent but not at 40-percent sugar concentration. An abundance of growth was noted at the 5- and 10-percent concentrations, as evidenced by the sediment at the bottom of the tubes and the color change of the medium, Bromothymol blue was the indicator used. At the higher concentrations growth decreased with increasing concentration. No growth appeared at the 40-percent concentration.

To account for the rapid disappearance of the fire blight bacteria during the process of elaborating nectar into honey several experiments were tried.

TABLE 2.—*Summary of studies conducted for the purpose of finding the maximum concentrations of various sugars, and of synthetic nectar, at which the fire blight organism, Erwinia amylovora, will grow*

Series no. and sugar	Growth of *E. amylovora* at the end of 10 days in a synthetic medium containing sugars in the indicated concentrations [1]											
	0 percent	2 percent	5 percent	10 percent	15 percent	20 percent	25 percent	30 percent	35 percent	40 percent	45 percent	50 percent
Series 1:												
Dextrose	−	+	+	+	+	+	+	+	−	−	−	−
Levulose	−	+	+	+	+	+	+	+	−	−	−	−
Sucrose	−	+	+	+	+	+	+	+	+	+	+	+
Nectar	−	+	+	+	+	+	+	+	+	−	−	−
	0 percent	3.75 percent	7.5 percent	15 percent	18.75 percent	22.5 percent	26.25 percent	30 percent	35 percent	40 percent	45 percent	50 percent
Series 2:												
Dextrose	−	+	+	+	+	+	+	+	−	−	−	−
Levulose	−	+	+	+	+	+	+	+	−	−	−	−
Sucrose	−	+	+	+	+	+	+	+	+	+	+	+
Nectar	−	+	+	+	+	+	+	+	tr.	−	−	−
	0 percent	5 percent	10 percent	20 percent	25 percent	28 percent	30 percent	32 percent	35 percent	40 percent		
Series 3:												
Dextrose	−	+	+	+	+	+	+	−	−	−	------	------
Levulose	−	+	+	+	+	+	+	−	−	−	------	------
Nectar	−	+	+	+	+	+	+	+	+	−	------	------
	0 percent	5 percent	10 percent	20 percent	30 percent	40 percent	48 percent	50 percent	52 percent	54 percent	56 percent	58 percent	60 percent
Sucrose	−	+	+	+	+	+	+	+	+	+	+	+	−

[1] + Growth; − No growth. The synthetic medium was made according to the formula given by the Society of American Bacteriologists (*14*).

The effect of desiccation alone on the longevity of the bacteria in sugar solution cannot account for their rapid disappearance in the honey-ripening process. A contaminated sucrose solution of 60-per-

cent concentration was placed in Petri dishes in a desiccator ($CaCl_2$) and kept there until the concentration of sugar was between 79 and 80 percent. The dishes were sealed and placed in a 30° C. incubator and were removed only for periodic examination. It was found that when 10, 20, 30, or 40 cc of the solution was placed in four Petri dishes of equal size the time required for the desiccator to adjust to the desired concentration was approximately 1, 2, 3, and 4 days. At the end of 1 month, when the isolation trials were discontinued, the bacteria were still being recovered from all the plates except the second. In this plate, the bacteria were still present at the end of 3 weeks. Portions of the original sugar solution were simultaneously incubated at the temperatures 6°, 12°, 18°, 24°, and 30°. At the end of 1 month the bacteria were still alive at 6° and 12°, but had disappeared at 18°, 24°, and 30° by the end of 18, 12, and 6 days, respectively.

When the experiment was repeated with sucrose solution, synthetic nectar solution, and similarly diluted honey of 21.2-percent sugar concentration as the starting point, comparable results were obtained. The rate of disappearance of the bacteria from these solutions slightly greater than for the sucrose solution, but cannot account for the rapid disappearance within approximately 2 days of the bacteria found in the feeding experiments detailed earlier.

An abundant microflora was found to be associated with the honeybee and the hive interior. Although these bacteria outlive the fire blight organism in honey, they too, disappear at the end of approximately 1 week. Preliminary studies on antagonism between these bacteria and the fire blight organism when grown on agar and in broth gave some indication that the former may function in some way in inhibiting the latter, but the evidence is too limited to justify its being given in detail.

THE FIRE BLIGHT ORGANISM AND THE LIFE CYCLE STAGES OF THE INSECT

Attention was next directed to the possibility that the bacteria might be associated with honeybees in some stage of the life cycle other than that of the active adult.

Two series of experiments were conducted in October 1933 and 1934. The same colonies of bees were used as in experiments 1933b and 1934b, already discussed. Shortly after the beginning of the feeding periods the queen bees, in response to the influx of food, began to deposit eggs in the cells, and continued to do so throughout the feeding period and for a short time afterward. As soon as the eggs hatched (3 days), the young larvae obtained their nourishment from a contaminated sugar solution or from the elaborated product, the only food available, and food of a contaminated source continued to be their diet until pupation (to the 9th day). In 21 days, or after 12 days as pupae, the adults emerged.

For making isolation studies from the immature life-cycle stages of the honeybee, small test tubes (1 by 6 cm) were fitted with cork stoppers into which round, stiff, toothpicks had been inserted. This type of pick was rigid and suited for loosening and removing specimens from the cells.

A frame containing brood was removed from the hive and the specimens were taken and stored in the test tubes for transporting to

the laboratory. A sterile scalpel was used for opening capped cells. For making isolations all specimens except the eggs were placed in a Petri dish containing a small amount of sterile water, chopped fine, and allowed to stand for about 10 minutes. A large loopful (6 mm) of the material was then transferred to broth and the usual procedure followed. In the isolations from the eggs a modified procedure was used. An egg was first allowed to stand for 10 minutes in water. After sampling the washings for the presence of the bacteria, the egg was crushed and handled in the same manner as the other stages. Several parallel isolation series were run in which the insect stages were placed directly in the broth.

In a preliminary experiment during the latter part of the feeding period in 1933, two series of isolations were attempted from, respectively, 10 eggs and 10 small larvae. One of the larvae in the first series yielded fire blight bacteria while the others were negative. Though limited in scope, this study indicated that the larval stage was receiving food containing the bacteria, although it is not known just when in the process of elaborating honey it was given to the larvae.

In the 1933 experiment the isolation intervals were 12, 14, and 17 days after the contaminated food had all been consumed, and in the 1934 experiment the isolation intervals were 15, 17, and 19 days. Both in 1933 and 1934, bacteria other than the fire blight organism were frequently obtained from the larval stage, but relatively rarely from the egg, pupal, and unemerged adult stages. The fire blight bacteria were not recovered in any instance in the regular series of tests after an interval of at least 12 days after the contaminated food had been taken, which is strong evidence that these bacteria are not associated with the life-cycle stages of the insect.

DISSEMINATION OF FIRE BLIGHT BACTERIA FROM THE BEEHIVE TO FLOWERS

The dissemination of *Erwinia amylovora* from the beehive to flowers caged with honeybees has been studied by several investigators. Gossard (*2*) postulated that if one bee carries 100,000 bacteria into the hive 1 day, on the following 1 or 2 days each of 1,000 bees has the potentiality of carrying a considerable fraction of 100 virulent bacteria out to fruit blossoms. He postulated further that the bacteria may multiply in the raw nectar when first carried into the hive. Pierstorff and Lamb (*10*), in studies conducted in Ohio, atomized the interiors of beehives with bouillon cultures of the fire blight organism, and concluded that: "Bees did not carry blight inoculum from the hive to the blossom clusters" when "beehives that had been infested with virulent cultures of *Bacillus amylovorus* were placed under apple trees enclosed within cheesecloth cages." However, they did get a small amount of infection possibly originating from the hive, which they attributed to factors incidental to the use of cheesecloth tents, such as rents or tears and splashing rain from diseased trees nearby. Thomas and Vansell (*19*) working with caged pears in California, found that when beehives were transferred from the diseased to healthy trees, no infections resulted. The methods of handling the bacteria may in part account for the discrepancies in these results.

Since it was desired to control the experimental conditions as closely as possible, small cages were used that fit over individual branches containing between 100 and 300 blossom clusters (fig. 2). The frame consisted of chicken wire of 1-inch mesh and 4 feet wide. A strip about 6½ feet long was folded to make a cylinder 4 feet long and approximately 2 feet in diameter. Wire screen and two types of transparent waterproof materials, consisting of waterproof cellophane impregnated over cloth and wire screen, were used as covers. For closing the cages over the branches, strips of unbleached muslin of about 40 meshes to the inch and 2 feet wide were sewed to the cages at either end. Ventilation was provided by inserting two pieces of wire screen 6 inches square into the cloth at either end, the inside one on the upper and the outside one on the lower side. Two strips of lath made to fit into the wire mesh of the support were used at either end of the cage for spreading the cage and transferring its weight to the branch. The nucelus hive of bees was suspended from the limb at the end of the cage nearest the tree.

The nucleus hives, unless otherwise specified, were made up to include: (1) Two feeders filled with sucrose solution, either sterile or contaminated with a water suspension of the fire blight bacteria; (2) one frame with brood ready to emerge; (3) one frame of sealed honey for emergency food supply; (4) one frame with empty comb to serve as a depository for pollen and for honey produced from sugar solution and nectar; and (5) 2 cupfuls of bees (approximately 500).

The cages were installed between the pink and early bloom stages of unfolding of fruit buds and were kept on until petal fall was completed and sufficient time had elapsed for disease symptoms to appear. Before installing the cages, as detailed earlier in experiment 1933s, special precautions of spraying were taken to control insects that might interfere with the experiment. The bees and food involved each season were from the same sources, except when specified otherwise, and were as uniform as possible. Bees from the same hives were used for the three seasons. To insure setting of fruit in the final of the 1935 series, a bouquet of McIntosh, a suitable pollenizer, was used to pollenize Rhode Island Greening blossoms in each cage containing bees.

Oozing cankers were prepared by inoculating succulent Bartlett pear shoots in the greenhouse and transferring these shoots when oozing copiously to flasks containing water which were suspended among the blossoms. To keep the interior temperatures of the rainproof cages near those outside, the cages were placed on the shady side of the trees and shade was provided when necessary.

In addition to investigating the transmission of the fire blight bacteria from the beehive to apple blossoms, studies were made of related aspects. For example: (1) The honey produced from contaminated food in the fall of the year was fed to a nucleus hive of bees housed in one of the enclosures to further check on the possibility of bacteria overwintering in the hive. There were also used bees from the same colony that had produced this honey. (2) As a check on (1), another hive with similar bees was fed sterile sugar solution alone; (3) the relation of bees to the oozing canker or overwintering source of fire blight inoculum was tested by caging bees with an

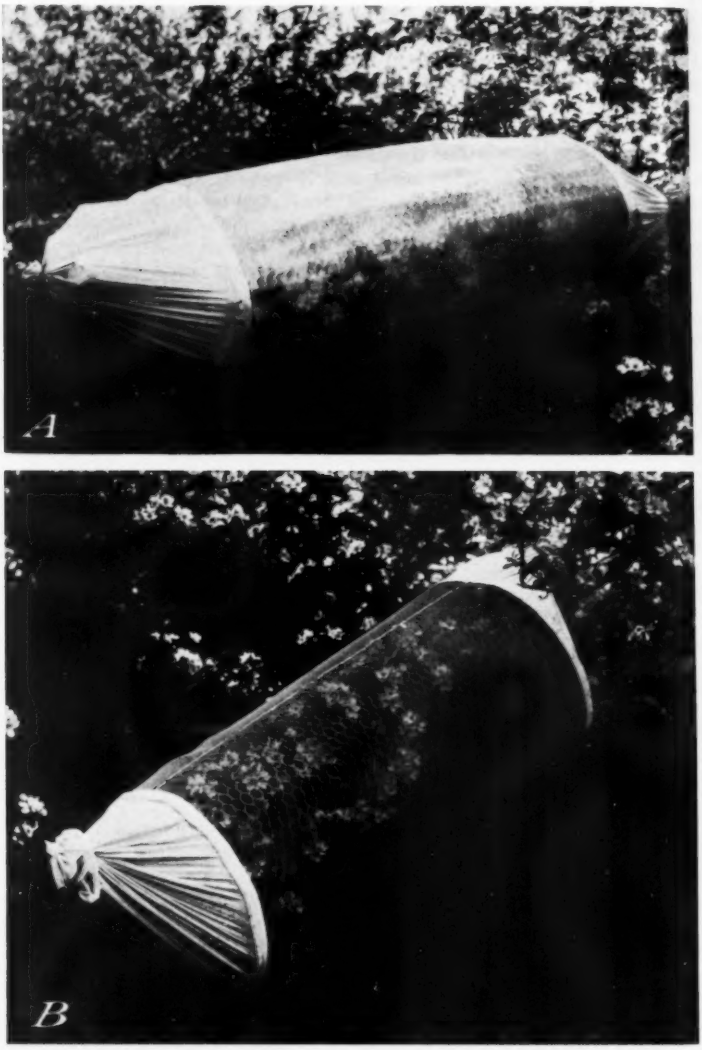

FIGURE 2.—Special cages for the study of the factors involved in the dissemination of the primary and secondary inoculum of fire blight: *A*, A waterproof cage designed to exclude such factors as insects and rain from the enclosed branch, used in studies of several factors such as insects, insect food, and cankers, alone or in combination; *B*, a screen-covered cage designed to exclude pollinating insects but to permit the entrance of rain, and adapted for studying the factors of rain, insects, and cankers in various combinations.

oozing canker; (4) a check on (3) was made by placing an oozing canker alone in a cage; (5) rain as a dissemination factor was tested by enclosing an oozing canker in a screen cage which permitted the access of rain; (6) a check for rain alone was employed; and finally (7) bees plus an oozing canker were enclosed in a screen cage admitting rainfall. These studies are summarized in table 3.

TABLE 3.—*Results of cage experiments conducted in the orchard to determine the role of the honeybee in the overwintering, primary spread, and secondary spread of the fire-blight organism* [1]

Factors studied	Year	Blossom clusters				
		Total	Healthy	Blighted		Setting fruit [2]
		Number	*Number*	*Number*	*Percent*	*Percent*
Bees + old honey made from contaminated food in preceding October	1933	214	214	0	0.0	
	1934	175	175	0	.0	
	1935	199	199	0	.0	32.0
Bees + contaminated sucrose solution	1933	219	205	14	6.3	
	1934	202	200	2	1.0	
	1935	204	152	52	25.5	30.0
Bees alone, check	1933					
	1934	210	210	0	.0	
	1935	204	204	0	.0	28.0
Rain alone, check	1933	112	105	7	6.3	
	1934	168	168	0	.0	
	1935	176	176	0	.0	.0
Canker alone, check	1933					
	1934	244	244	0	.0	
	1935	162	162	0	.0	.0
Bees + oozing canker	1933	211	211	0	.0	
	1934	171	171	0	.0	
	1935	151	151	0	.0	18.0
Oozing canker + rain	1933	208	191	17	8.2	
	1934	219	219	0	.0	
	1935	131	129	2	1.5	.0
Bees + oozing canker + rain	1933	215	4	211	98.1	
	1934	202	201	1	.5	
	1935	158	155	3	2.0	25.0

[1] The cages were installed and removed on the following dates: In 1933, May 13 and 26; in 1934, May 18 and 29; in 1935, May 22 and June 3. All the cages, except where the factor for rain was involved, when a screen cage was employed, were made of a transparent waterproof material. Rainfall during the experimental periods was in 1933 on May 13, 0.06 inch, May 20, 0.42 inch and May 24, 0.52 inch; in 1934 on May 22, 0.01 inch and on May 24, 0.09 inch; and in 1935 on May 28, 0.09 inch and May 29, 0.03 inch.

[2] Pollenizers were introduced into the cages only the last year, but in the preceding 2 years as well as in the last year a record was kept of the visits made by the bees to flowers for time intervals of 5 minutes on succeeding days. These data, although not included here, show that the bees visited flowers in all cages.

Blossom blight developed in cages where the bees were fed freshly contaminated sucrose solution. This is evidence that the bacteria were carried from the beehive to the flowers to bring about the infection, since no other known source of the bacteria was available. However, it is not possible to state how many bees were involved, or whether the sucrose solution had first been concentrated. It is probable that certain of the 1935 infections resulted from secondary spread by the bees after some bacteria had been carried out of the hives to the flowers, for from June 3 to June 9 the number of blighted clusters increased from 43 to 52, or approximately 20 percent.

No blossom infections occurred in the cages containing honeybees and honey for food that had been produced the previous autumn from contaminated sucrose or honey solution. These data confirm the results of the isolation experiments in which it was found that the fire blight bacteria quickly disappear from honey elaborated from heavily contaminated food.

Cage experiments to check the possibility that the bacteria may overwinter in the bees themselves gave negative results. The bees used in these experiments had consumed contaminated food the previous fall and were given sterile sucrose solution while in the cage. These results confirm those of the experiments in which it was found that the bacteria were not associated with the life-cycle stages of the insect.

No blossom infections resulted from caging bees with oozing cankers. This finding is in conformity with the observations of a number of workers, including Jones (*6*) and Thomas and Parker (*18*). Apparently honeybees are not attracted by the bacterial ooze of the fire blight disease. In similar cages containing only the oozing cankers, there were likewise no infections.

Rain was found to be a factor in the dissemination of fire blight bacteria from oozing cankers to the neighboring blossoms. In 1933 the rainfall, which approximated 1 inch during the experimental period, was sufficient to cause the infection of 8.2 percent of the caged blossoms. The blighted blossom clusters were all in the immediate vicinity of the oozing canker. Subsequent trials in 1934 and 1935 resulted in practically no infection because of the lack of sufficient rainfall while cankers were in the cages, it being only 0.10 and 0.12 of an inch for these years.

In the check for rain alone for 1933, 6.3 percent of the blossom clusters were blighted. These infections were traced to a canker in the tree above and slightly to one side of the cage. In the 2 succeeding years, no infections were encountered in similar experiments.

When the factors of oozing canker, rain, and bees were combined in the same cage, the percentage of infected blossom clusters for the three seasons were, respectively, 98.1, 0.5, and 2.0. The rainfall was sufficient only in 1933 to disseminate some of the bacteria from the oozing canker to the flowers in the primary spread of the organism. The large percentage of blossom clusters infected demonstrates the role of the honeybee as a secondary spreader of the bacteria from flower to flower. The isolated infections in 1934 and 1935 may be accounted for by the possible direct contact between blossoms and oozing canker because of their close proximity. The results of these experiments on the role of the honeybee as a disseminator of secondary inoculum are in agreement with the findings of Waite (*20*), Gossard and Walton (*4*), Thomas and Vansell (*19*), and Pierstorff and Lamb (*10*).

From these data, it appears that the honeybee may function in two ways,[7] namely, (1) in carrying the bacteria from the beehive to flowers, and (2) in the secondary spread of fire blight inoculum from flower to flower. The transmission of inoculum in the first instance is conditioned, however, on the bees actively feeding on food contaminated with the fire blight organism. It would seem probable during years of blight epiphytotics that the causal bacteria may be carried out from the hive and initiate infections, with the insect apparently functioning purely in an accidental capacity. These data confirm the results of the isolation experiments discussed earlier in which bees feeding on contaminated food harbored the organism for

[7] The honeybee may also disseminate the bacteria in another way. Unpublished data show that the causal bacteria may infest pollen in blighting flowers even before anther dehiscence. Pollen-gathering bees carry such pollen from flower to flower and infection results when it lands on the stigmas of flowers, even when the relative humidity of the surrounding atmosphere is extremely low.

no longer than 1 day. The percentage of bees functioning in this capacity was relatively small (table 1), which would seem to indicate that the hazard of infections from this source is not great.

SUMMARY

The results of studies to determine the role of the honeybee and beehive in the development of the fire blight disease indicate that the fire blight organism is incapable of overwintering in the beehive or in association with the honeybee. Therefore these locations cannot constitute a source of primary inoculum in the spring of the year.

This investigation extended over a period of four seasons and had several objectives, namely, (1) to discover the longevity of the bacteria causing fire-blight in the beehive when introduced through the natural channel in the food of the bees, (2) the longevity of the bacteria in sugar solutions over a range of concentrations and temperatures, (3) the location of the bacteria in relation to the life cycle of the insect, and (4) the extent of dissemination of the bacteria from the beehive to flowers in cages in the orchard.

When introduced through the food of the bees, the fire blight bacteria were not reisolated after 3 days from such locations as honey, comb, frame, and bees. Only in two instances were the bacteria recovered after 3 days from the time the contaminated food was taken, that is, from pollen at 13 days and from frame scrapings at 12 days.

The longevity of the bacteria in the sugar solutions varied with the incubation temperature, the sugar concentration, and the sugars used. These data provide evidence that the survival of the bacteria in the beehive is highly improbable at the temperatures and sugar concentrations normally encountered in the apiary. The bacteria survived for relatively short intervals (from 1 day to several weeks, depending on the kind of sugar and the incubation temperature) at sugar concentrations of 30 percent and above, covering the range of heavy nectar to honey that would be found in the beehive. The bacteria ordinarily survived longest at the lower temperatures. Of the materials used, sucrose solution had the least bacteriostatic and bactericidal effect on the bacteria. When a synthetic culture solution was used as a base, the maximum sugar concentrations at which the fire blight organism grew in dextrose, levulose, artificial nectar, and sucrose were, respectively, 30, 30, 35, and 58 percent.

The fire blight bacteria were not found associated with the immature stages of the life cycle of the honeybee even though an abundance of the bacteria were present in the food of the bees at all times during the rearing of the brood.

The honeybee was found to function in two ways, namely, (1) in carrying the bacteria from the beehive to flowers, and (2) in the dissemination of fire blight inoculum from flower to flower. Transfer of inoculum from the beehive to flowers was conditioned, however, on the bees actively feeding on contaminated food.

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